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REVIEW ARTICLE

The Role of Lipids and Membranes in the Pathogenesis of Alzheimer's Disease: A Comprehensive View

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Abstract: Lipids participate in amyloid precursor protein (APP) trafficking and processing – important factors in the initiation of Alzheimer's disease (AD) pathogenesis and influence the formation of neurotoxic β-amyloid (Aβ) peptides. An important risk factor, the presence of ApoE4 protein in AD brain cells binds the lipids to AD. In addition, lipid signaling pathways have a crucial role in the cellular homeostasis and depend on specific protein-lipid interactions. The current review focuses on pathological alterations of membrane lipids (cholesterol, glycerophospholipids, sphingolipids) and lipid metabolism in AD and provides insight in the current understanding of biological membranes, their lipid structures and functions, as well as their role as potential therapeutic targets. Novel methods for studying the membrane structure and lipid composition will be reviewed in a broad sense whereas the use of lipid biomarkers for early diagnosis of AD will be shortly summarized. Interactions of Aβ peptides with the cell membrane and different subcellular organelles are reviewed. Next, the details of the most important lipid signaling pathways, including the role of the plasma membrane as stress sensor and its therapeutic applications are given. 4-hydroxy-2-nonenal may play a special role in the initiation of the pathogenesis of AD and thus the "calpain-cathepsin hypothesis" of AD is also highlighted. Finally, the most important lipid dietary factors and their possible use and efficacy in the prevention of AD are discussed.

Keywords: Lipids, membranes, Alzheimer's disease, lipids in Aβ aggregation, lipid-protein interactions, 4-hydroxy-2- nonenal, ApoE, membrane lipid therapy, lipid dietary factors.

1. INTRODUCTION

In the central nervous system (CNS) of several neurodegenerative diseases (NDDs) misfolded, toxic protein aggregates (amyloids) are accumulating. These protein assemblies have no physiological functions. The neurons are the most vulnerable cells of the CNS [1]. The presence of the toxic protein assemblies indicates the serious disturbance of protein homeostasis in the CNS. Different cell types may possess big differences in the effectivity of the proteostasis network [2]. Although the mechanism of the special vulnerability of neurons is not well understood, very probably the dysfunction of the proteostasis process plays a key role in neuronal death [3].

Alzheimer's disease (AD) is the most common form of dementia starting with synaptic dysfunction and decrease of dendritic spines [4]. Many cellular processes show dysfunction in AD brain cells (energy metabolism in mitochondria, protein folding in endoplasmic reticulum (ER), etc [5]. According to the oldest and widely accepted hypothesis of AD, the accumulation of an aggregation-prone polypeptide, the toxic β-amyloid (Aβ) assemblies may initiate the toxic cellular processes [6]. The formation of Aβ aggregates starts within the cells in a very early stage of the disease [7]. Both the intra- and extracellular Aβ may be neurotoxic. AD is a heterogeneous disease with several different subtypes [8].

Amyloid precursor protein (APP) has two main processing pathways: 1) the non-amyloidogenic pathway (90%) involving α-secretase and 2) the amyloidogenic pathway (10%), which generates Aβ peptides through the sequential proteolytic cleavage by β-secretase (BACE) and γ-secretase (Fig. **1**). The non-amyloidogenic cleavage of APP is running in the plasma membrane [9], the amyloidogenic cleavage occurs intracellularly [10]. The main cleavage products of the amyloidogenic pathway are 40 and 42 amino acid peptides (Aβ1-40 and Aβ1-42). Recent analytical studies demonstrated the presence of longer (Aβ1-43), and shorter frag-

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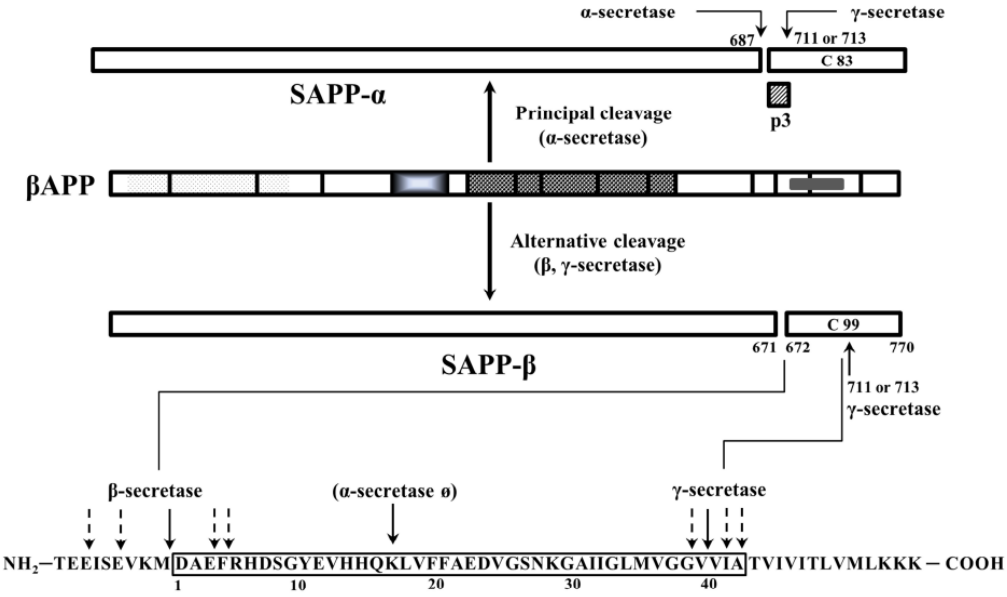

Fig. (1). Schematic representation of APP with the two main proteolytic pathways and the processing products.

ments (Aβ1-38, Aβ1-34) as well as tri-, tetra-, penta- and hexapeptides after stepwise successive processing of the polypeptide chain [11-13].

Tau is another aggregation-prone protein playing a role in AD pathogenesis [14]. A direct interaction of Aβ and hyperphosphorylated tau (pTau) has been shown; however, the pathological role is unknown. Although they are interconnected, the appearance of intracellular Aβ precedes the presence of pTau in cells.

A Alzheimer found "adipose inclusions" or "lipoid granules" in AD-brain tissue. This was the third hallmark of the disease besides amyloid plaques and neurofibrillary tangles [15]. Recent findings demonstrate that brain lipids and lipid membranes play an important role in the proteolytic cleavage of APP to Aβ peptides and their aggregation to toxic Aβ assemblies. Membrane lipids participate in the lethal pathophysiological pathways leading to neuronal death from the beginning. The cholesterol content of the membrane rafts affects the amyloidogenic processing of APP and, in the next step, cholesterol and the ganglioside GM1 may initiate the formation of toxic Aβ oligomers. Changes in cholesterol metabolism and trafficking (related to late AD susceptible genes) may result in a decrease of Aβ clearance and initiate AD [16].

These findings demonstrate that both protein- and lipid-homeostasis in the brain play a role in the pathophysiological processes leading to AD.

Brain lipids consist of three main classes that are present in roughly equimolar amount: glycerophospholipids (GPs), sphingolipids, and cholesterol. Alterations in the lipid homeostasis may be involved in the pathomechanism of AD [17]. Changing of lipid composition in raft structures (Section 2.2.) and microenvironments occurs in AD [18]. According to a novel hypothesis ("membrane aging"), the functional and structural alterations of membranes represent the initial pathogenic factor for AD [19]. Genetic evidence also indicates the importance of lipids in AD pathomechanism. Human genome-wide association studies (GWAS) of late onset AD patients show that a lot of AD risk genes (APOE, ABCA1, ABCA7, SORL1, PICALM, BIN1) and their gene products are involved in lipid metabolism and transport [16, 20]. For revealing the pathomechanism of AD, it is necessary to understand the structure and role of biological membranes which is the actual place where the proteolytic cleavage of APP to Aβ and formation of toxic Aβ assemblies occur.

2. MEMBRANE STRUCTURE, MEMBRANE LIPIDS, MEMBRANE MICRODOMAINS

Biological membranes form selective barriers that constitute the boundary of the cell as well as of subcellular organelles. The functions of the membranes depend both on particular membrane proteins and lipid composition, as well as their interactions defining the formation of membrane nanostructures [21, 22]. The knowledge of membrane structure and function provides a solid basis for understanding the pathophysiological pathways of AD and similar diseases.

2.1. The Basic Structure of Biomembranes

The lipid bilayer structure of the cell membrane was already proposed in 1925. The fluid mosaic membrane model (F-MMM) was the first hypothesis that took into account the mobility of membrane components and their dynamics [23]. This model described biomembranes as fluid bilayers of phospholipids with mobile integral membrane proteins and glycoproteins that were intercalated into the lipid bilayer. This model depicted the dynamic changes of the membrane structure and the continuous mobility of its components: lipids and integral and peripheral membrane proteins (Fig. **2**).

Although the original F-MMM is still valid, certain modifications of the original model have been performed [22, 24, 25]. According to our present knowledge, biomembranes have a high density of transmembrane proteins. Sev-

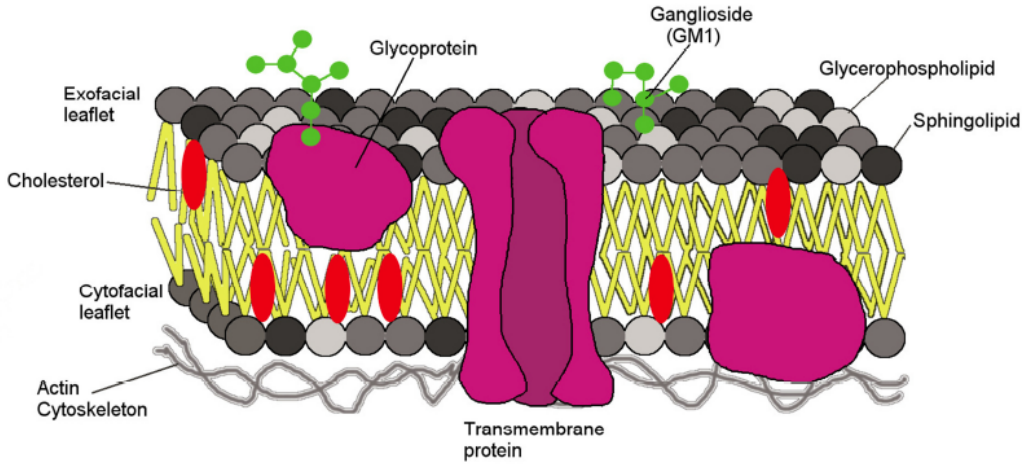

Fig. (2). The simplified structure of biomembranes according to the fluid-mosaic membrane model.

eral proteins bind to the membrane occasionally, localized both in the cytosol and in the membrane. The mobility of cell membrane components can be restricted by association to cellular active fibers or indirectly to microtubular structures. Membranes show lateral heterogeneity in which special microdomains ("patches") may be formed with diameters between 10 to 200 nm in size around integral membrane proteins and glycoproteins which are enriched in certain specific lipid clusters, the so-called membrane rafts [26]. As each cell compartment has a specific lipid profile, the lipid composition of different organelles is different. Lipid organization is tightly regulated and probably evolutionarily conserved. Table **1** shows the major lipid classes and their role in the brain.

Table 1. Major lipid classes and their role in the brain.

Lipids	Functions
Cholesterol	Precursor of lipid mediators and hormones, major component of cellular membranes, crucial role in the formation of lipid rafts.
Glycerophospholipids	Precursors of lipid mediators, important component of neural membranes for stabilizing structural integrity; participation in oxidative stress and neuroinflammation.
Sphingolipids	Crucial role in structural integrity in neural membranes, precursors of lipid mediators, formation of lipid rafts, participation in oxidative stress and neuroinflammation.

2.2. Lipid Rafts

Biological membranes are "a meeting point for lipids, proteins and therapies" [27]. Membranes are composed of dynamic lipid and protein clusters referred to as microdomains. Although the amino acid sequence determines the membrane topology of proteins, the composition of these microdomains - anionic phospholipids, cholesterol, sphingomyelin, and membrane fluidity (see Section 7.2) - as well as post-translational modifications (*e.g.* phosphorylation, acylation) also play an important role in the topology [28]. During the last 20 years, a special type of lipid domains, the lipid raft has been in the center of interest. Rafts are small (10-200 nm), short-lived (~ 100 msec), membrane microdomains with special physiological functions. Rafts are enriched in cholesterol and sphingolipid content. Lipid rafts are concentrating platforms for essential cell-signaling receptors and can influence intracellular signaling. Receptor activation by ligands may occur in the rafts and the signaling complex is protected from non-raft enzymes, such as phospholipid phosphatases (PLPP) [29]. Caveolae, special membrane invaginations with particular lipid and protein composition, are formed by the polymerization of caveolins and participate in the endocytosis of different proteins (*e.g.* albumin) and in signal transduction. Lipid rafts participate in the pathophysiology of degenerative diseases, such as atherosclerosis [30].

3. METHODS FOR STUDYING THE MEMBRANE STRUCTURE, LIPID LEVEL AND COMPOSITION, AND AB-MEMBRANE INTERACTIONS

As key pathogenic molecules of AD (Aβ-peptides, tau proteins) interact with membranes [31, 32], there is a need for advanced techniques to examine membranes in the presence of these toxic molecules. Moreover, as constituents of the membrane (*e.g.* sphingolipids such as the ganglioside GM1 as well as cholesterol) can also control the cytotoxicity of AD related molecules, a determination of lipid composition is essential [33]. Lipid changes in blood plasma might be applied as a diagnostic tool for AD (section 4.5).

Focusing first on experimental analytical techniques, NMR is one of the most commonly used tools to determine the changes in membrane structure induced by the presence of toxic peptides. Solid-state deuterium and phosphorus-31 NMR measurements were applied in combination with selectively deuterated lipid molecules in the presence of Aβ-peptide by Terzy at al. [34]. Although electron spin resonance (ESR) is not a frequent technique, it was still used in certain cases in combination with circular dichroism (CD) measurements [35]. Obviously, CD was applied primarily to examine the secondary structure elements of the interacting toxic peptide. However, in most cases, the combination of different techniques is necessary to have a detailed insight into the lipid-membrane complexes. For example, Lashuel *et al.* used the combination of electron microscopy, CD, NMR

and multi-angle light scattering (MALS) experiments to determine the structure of toxic tau/phospholipid oligomeric complexes [32].

Mass-spectrometric (MS) methods are very popular to determine the lipid contents of different tissues and bodily fluids. An outstanding summary of MS methods applied in lipidomics can be found in [36]. Nowadays, the two most common techniques are used to analyze lipids, namely MS-based analysis with direct infusion, called the "shotgun" method, and liquid chromatography coupled to mass spectrometry (LC-MS). The main advantages of the application of hyphenated techniques compared to direct infusion methods are their ability to reduce signal suppression and to distinguish lipid isomers. However, the shotgun technique provides higher throughput and avoids difficulties with abnormal chromatographic behaviors of given lipid species. Recently, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging has become a tool of choice for the analysis of endogenous lipids in AD related lipidomics studies (see Table **1** in [37]). Overall, lipidomics methods enable to follow the rapid changes of lipids in pathological processes.

Concerning microscopic techniques, transmission electron microscopy (TEM) have been used continuously in membrane structure investigations since the 1950s. In the last decade [38], up-to-date X-ray scattering microscopic techniques (SAXS, WAXS) also gained more ground. Finally, atomic force microscopy (AFM) as another scanning techniques can also help to clarify the structural background of lipid-membrane interaction and how it depends on membrane composition [39].

Recently, Brameshuber and co-workers presented a special photobleaching method using fluorescent marker proteins that allowed the direct imaging of rafts in the live cell plasma membrane [40].

Although all these methods can reach high-level resolution, it is very difficult to ensure exact circumstances in experiments owing to the continuously changing structure of Aβ oligomers. Therefore, a more advanced picture can be reached with the help of computational simulations regarding Aβ-membrane interactions. Because of the enormous progress in hardware and software development, all-atom explicit molecular dynamics (MD) simulations can provide valuable information about the Aβ monomer/oligomer-membrane system.

MD simulation software such as Gromacs [41], NAMD, Amber [42] or Desmond [43] are capable of considering many types of lipid components such as different glycerophospholipids sphingomyelin, or cholesterol. Currently developed atomistic force fields (FF) can model lipid bilayers in good agreement with experimental results. The widely used FF have been GROMOS96 53A6, l CHARMMc36 [44], OPLS-AA or Lipid14 [45] in these calculations, and the starting membrane-protein complexes can be built via CHARMM-GUI [46] or VMD membrane builder [47]. It is important to know that while CHARMM and GROMOS force fields contain all type of lipid components, the available lipid components within LIPID14 or OPLS force fields are much more limited. If the principal aim is to build a complex membrane system, the CHARMM or GROMOS force fields is a reasonable choice in combination with the application of the GROMACS package. However, it should be remembered that these simulations are not trivial MD calculations because there are no automated system preparation methods; therefore more experimental results are needed. Finally, we would like to mention that although all these MD packages are very well optimized concerning the graphics processor usage, the Amber or Desmond code require considerably less processor power. Therefore, the usage of these latter codes in a single workstation or small cluster with many graphics processor cards but with limited processor cores is a good choice.

Interestingly, recent computational experiments focusing on Aβ/membrane complexes calculated that the pores of the Aβ channel structures can modify the water and ion flux across the membrane [42, 48]. Another study simulated how the membrane rafts can affect the binding and dimerization of Aβ peptides [43]. Also, MD simulation shows that cholesterol has a protective effect on the dipalmitoyl phosphatidyl choline bilayer structure against bilayer thinning caused by Aβ binding [49].

4. THE ROLE OF LIPIDS AND LIPID MEMBRANES IN AD

4.1. Alteration of Lipids in AD

Dysregulation of lipids and lipid-mediated signaling pathways has been found in several NDDs such as AD, the recent results have been widely reviewed by G. Di Paolo and T-W. Kim [50].

4.1.1. Cholesterol

The human brain contains 23% of the body's total cholesterol. Both neurons and astrocytes are capable to produce cholesterol. However, strong evidence showed that a large pool of cholesterol in the brain is produced by astrocytes (and microglia) that is delivered to neurons via lipoproteins [51]. The strict regulation of cholesterol production is crucial for cerebral cholesterol homeostasis. Thus, cholesterol is removed from the brain by enzymatic conversion to 24S-hydroxycholesterol, which readily crosses the blood-brain barrier (BBB) [52].

The association between plasma/serum cholesterol and AD is not clear. High plasma cholesterol levels might be a risk factor for AD [53]. The hypothesis that cholesterol is a direct causative factor in AD has been debated [54]. Also, the use of statins for the treatment of AD has been ineffective [55]. Thus, there is conflicting epidemiological data in the literature: besides the above mentioned negative association [55], the high cholesterol level as a direct causative factor of AD was also reported [56]. Cholesterol may participate in the formation of toxic Aβ assemblies (section 4.4). These discrepancies of the results are likely due to different study design, age of participants, *etc*.

4.1.2. Fatty Acids and Oxylipins

The harmonious development of the brain, the neuronal plasticity, and cognitive performance is conditioned by several lipid nutrients. Essential fatty acids represent one of the most important groups. Polyunsaturated fatty acids (PUFAs)

such as linoleic acid (18:2, ω-3), linolenic acid (18:3 ω-3), arachidonic acid (20:4 ω-6) and docosahexaenoic acid (DHA, 22:6, ω-3) are the most important PUFAs in the brain. In fact, DHA accounts for 8% of the brains dry weight. The endogenous DHA synthesis is limited in the human brain, however, DHA enters the human brain across the BBB [10, 57]. DHA rapidly incorporates into phospholipids and increases membrane fluidity [58]. DHA is very sensitive to lipid-peroxidation during AD pathogenesis. It was found that the DHA content is reduced in the plasma and certain brain regions of AD patients. In addition, epidemiological analyses found that the dietary intake of DHA reduced the risk of AD (section 8.2) [59].

Other PUFAs are also very susceptible to reactive oxygens species (ROS) and lipid peroxidation. Oxylipins are formed in these oxidative reactions. Oxylipins (oxidized lipid mediators) are an important family of bioactive lipid metabolites, which are synthesized by the regulated oxidation of PUFA- precursors, mainly from arachidonic acid (ARA), DHA, eicosapentaenoic acid and dihomo-γ-linolenic acid. In the first step, PUFAs are generated by phospholipase A2 cleavage of phospholipids. Then PUFAs are metabolized to oxylipins through one non-enzymatic and three main enzymatic pathways, such as cyclooxygenase (formation of prostaglandins and thromboxanes), lipoxygenase (formation of leukotrienes and resolvins) and cytochrome P450 (formation of epoxides) pathways [60-62]. Considering the high amount of peroxidizable PUFAs, the brain is thus very sensitive for oxidative imbalance. Hence, oxidative stress might play a role in the pathogenesis of AD. The metabolites formed by oxidation may serve as biomarkers (section 4.5) [63, 64]. Indeed, numerous studies reported elevated levels of F2-isoprostane species, such as F4-neuroprostanes (F4-NPs) and F2 isoprostanes (F2-IsoPs) in cerebrospinal fluid (CSF) and brain tissue from AD patients. Also, increased levels of 8,12-iso-iPF2a-VI were found in brain, CSF, plasma and urine samples of AD patients [64]. Another study found increased levels of 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) in AD brains [65]. Oxylipins such as HNE and 4-hydroxy-hexenal increase Aβ level via increased gene expression of β- and γ-secretase and additionally by a direct action on β-secretase activity. Unfortunately, already 1% of oxidized DHA products (oxylipins, see further) reverts the inhibitory effect of DHA on Aβ production [66]. DHA should be protected from oxidation in nutritional approaches (Section 8.2.). Furman et.al demonstrated that the concentration of hydroxy-eicosanoid (HETE) species was elevated in AD frontal cortex, although, the level of more complex oxidation products of ARA was not changed significantly [67].

4.1.3. Glycero- and Sphingolipids

Phospholipids form cellular membranes and are involved in the activity of membrane proteins, receptors, enzymes, and ion channels both intracellularly and at the cell surfaces. There are structurally different classes of phospholipids. Glycerophospholipids are represented by phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidyl-choline (PC), as well as plasmalogens, such as ethanolamine plasmalogene (PPE). The big family of sphingolipids also includes the gangliosides with very complex structure. The chemical structure of the ganglioside GM1 is shown in Fig. (**3**).

Phospholipid composition of the different brain areas is different. Sensitive techniques, (*e.g.* mass spectrometry imaging, section 3) enable more precise, regional distribution profile of the brain phospholipid content [68]. GWAS investigations indicated that membrane phospholipid changes may be at the center of AD pathogenesis. Bioinformatic analyses demonstrated that genetic loci, relevant to late onset AD are implicated to lipid metabolism [6]. These AD-risk genes include, at a minimum, APOEε4, ABCA7, TREM2, and PLCG2. Analysis of the function of these genes hints to their role in AD pathogenesis. ABCA7 is a phospholipid transporter whereas TREM2 is a phospholipid receptor. PLCG2 itself is a phospholipase enzyme whereas ApoE is one of the most important phospholipid transporters. According to the newest hypothesis of Hardy: as the proteins (codified by these genes) are involved in the clearance of membrane damage around amyloid plaques, the liberation of membrane phospholipids might be the key event in AD pathogenesis [6].

Brain phospholipid changes occur both in aging and during the pathogenic processes of AD, (extensively reviewed in [69]). In fact, the concentration of most lipids decreases after the age of 50 year in the human brain. PI, PE, and PC levels decrease slowly with age. In contrast, PPE brain levels decrease by 18% till the age of 70 and 29% till the age of 100. There are significant changes in phospholipid brain

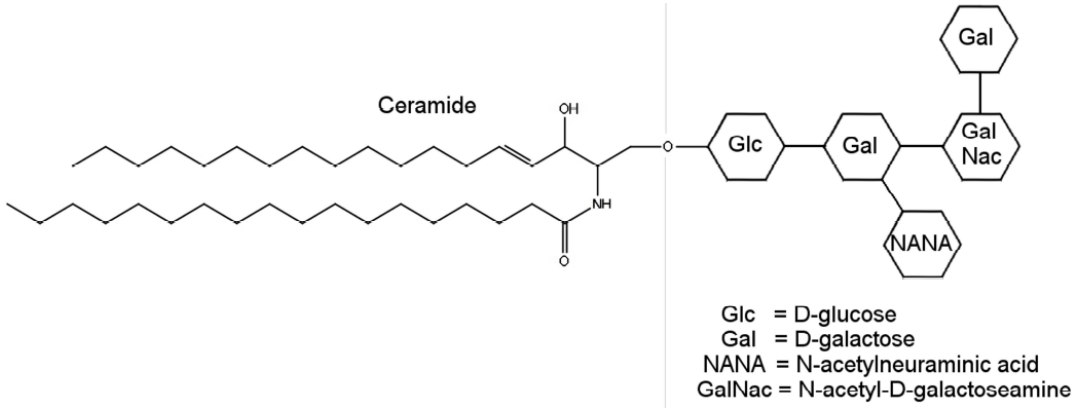

Fig. (3). Schematic drawing of the structure of the ganglioside GM1.

level of AD patients (decreased PI, PE and PPE levels) [70]. Deficiency of PPE both in white and grey matter is characteristic in AD [71].

Besides these changes, alteration in the brain sphingolipid levels also occurs during AD. Cell and animal studies suggest that sphingolipids contribute to AD pathology. Ceramide, the main precursor of sphingolipid metabolism, is increased in the cortical regions of AD brains. Sphingolipids (ceramides, sphingomyelins and sulfatides) are elevated in CSF of AD pathology. Elevated blood ceramide levels predict cognitive impairment and prodromal AD as well as memory decline [72]. Bioactive sphingolipids play critical roles in major cell biologic responses. This role emphasizes the importance of sphingolipid metabolism to several NDDs. Sphingolipids are the main factors of neurodevelopment and neurodegeneration in AD and traumatic brain injury [73]. A special group of sphingolipids, the complex gangliosides, are critical players in AD. It has been long known that defects of sphingolipid catabolism cause severe lysosomal dysfunction [74]. Aβ activates sphingomyelinase and thus increases ceramide and decreases sphingomyelin levels [75]. Sulfatide is the most affected sphingolipid species as it is depleted up to 58% in white and 93% in the grey matter very early in AD brain [76]. The changes in sphingolipid metabolism in AD is thoroughly reviewed in [77]. Sphingolipids are important structural components (one-third of the content of cell membranes is sphingolipid), and their metabolites are second messengers, modulating intracellular signaling [72].

4.2. Lipid Regulation of the Amyloidogenic Processing of APP

Although APP is a typical transmembrane protein of the plasma membrane, it can also translocate into the subcellular compartments. In steady state, APP is predominantly a Golgi localized protein. APP transport is highly dynamic involving transport in secretory and endocytic compartments. The amyloidogenic processing may occur in the early endosomes [86], lysosomes [80], as well as the mitochondria-associated membrane (MAM) [78, 79] where APP and β- and γ-secretases are co-localized [80]. The lipid composition of membranes influences the APP-processing pathways. Cholesterol depletion inhibits the generation of Aβ in hippocampal neurons [81]. Low cholesterol levels of membranes activate the non-amyloidogenic pathway by affecting the α-secretase ADAM10. Recent studies demonstrated that simvastatin (a known drug for inhibiting cholesterol biosynthesis) strongly reduced the levels of Aβ1-40 and 1-42 [82]. The fine details of the role of cholesterol in the amyloidogenic processing of APP were studied by [83]. Lovastatin-regulated cholesterol depletion inhibits β-cleavage by altering APP processing. β-Secretase is located in cholesterol-rich membrane rafts, the place for the amyloidogenic cleavage of APP. Within the cholesterol-poor membranes, the α-secretase hydrolyses APP and the subsequent cleavage by γ-secretase results in non-amyloid peptides.

The activity of ApoE protein links the cholesterol metabolism to AD. GWASs showed that the activity of APOEε4 alleles increases the risk of late onset AD. The ApoE protein has 3 isoforms (ApoE2, ApoE3, ApoE4) differing only in a single amino acid at residues 112 and/or 158. ApoE proteins play a major role in both intra- as intercellular transport of cholesterol and other lipids. ApoE4 protein is associated with higher, whereas ApoE2 and ApoE3 are associated with lower cholesterol levels. As a consequence, different isoforms have different effects on cholesterol transport and metabolism. The ApoE isoforms have different lipidation states and stability against degradation in the brain [84]. In neurons, ApoE proteins participate in the regulation of lipid metabolism, intracellular cholesterol transport and esterification as well as lipid efflux, in an isoform-specific manner [85]. As these transport proteins may be involved in the receptor-mediated endocytosis of APP (and other lipoproteins) in the brain, ApoE isoforms have a very complex role in the pathogenesis of AD. This endocytosis may result in the amyloidogenic processing of APP in early endosomes [86]. Recent studies linked ApoE with vascular function, microglia activation and neuroinflammation [87].

4.3. APP and Cleavage Peptides Regulate Lipid Metabolism – a Bidirectional Link Between APP and Lipids

There is a mutual interdependence between APP processing and lipid biosynthesis. The enzymatic cleavage of APP is strongly influenced by the lipid environment and the cholesterol content of membranes [10]. Inversely, the regulation of lipid metabolism is the physiological function of APP and some APP cleavage products. The APP is a regulator of cholesterol biosynthesis: full-length APP [88] and the APP C-terminal fragment [89] could interfere with cholesterol metabolism and transport. The flexible transmembrane domain of APP binds directly to cholesterol [90]. The cleavage product Aβ directly affects cholesterol synthesis and transport [91]. Aβ may also decrease cholesterol biosynthesis by inhibiting the enzyme HMG CoA reductase [92]. Aβ also affects the activity of sphingomyelinase thereby providing a link to the metabolism of sphingolipids [93]. Aβ peptides can modulate or interfere with the normal (lipid binding and transporting) function of ApoE [94]. The intracellular APP-domain (AICD) may act as a transcription factor to suppress production of the cholesterol transporter protein LRP1 [95]. AICD plays an important role in regulating lipid homeostasis. The complex bidirectional link between lipids and APP processing and APP-derived peptides in AD has been widely reviewed very recently [10].

4.4. Formation of Toxic Oligomeric Aβ and pTau Assemblies with Protein-Lipid Interactions

According to the amyloid hypothesis, the conversion of soluble, nontoxic Aβ to toxic β-structured Aβ assemblies is the key step in the initiation of AD. Increasing evidence indicates that Aβ-cell membrane interaction is a primary step in AD [96]. Aβ production and aggregation occur with interaction by membrane lipid rafts. As the composition of lipid membranes is changing during aging and in AD pathogenesis, this change may trigger the formation of toxic Aβ oligomers [97]. The presence of membrane cholesterol influences membrane fluidity and that leads to different aggregation pathways and Aβ-assemblies [98]. Several studies demonstrated that both GM1 and cholesterol are important factors determining Aβ aggregation in membrane environment.

Aβ peptides in the lipid raft region [99] preferentially bind to the ganglioside GM1 and cholesterol [100]. GM1 ganglioside tightly binds Aβ and this specific structure may act as a seed for the initiation of the formation of toxic Aβ assemblies [101]. Aβ may recognize cholesterol-dependent clusters of GM1-containing liposomes [102]. Although the fine details of the ganglioside GM1-Aβ interaction are unknown, the involvement of GM1 in Aβ aggregation was demonstrated [103].

Free cholesterol content in brain neurons increases the amount of intracellular Aβ-peptides. Cholesterol-rich lipid rafts may catalyze the conversion of monomeric Aβ-peptide to neurotoxic oligomers [104]. Aβ oligomers isolated from AD patients are found to be associated with the lipid microdomain in a cholesterol-dependent manner. A wide variety of biophysically and biochemically distinct Aβ-oligomer subtypes, the so-called different Aβ "strains" may parallelly exist [105]. Depletion of cholesterol was found to reduce Aβ aggregation to toxic oligomers. According to several experiments, cholesterol plays a critical role in the internalization of Aβ-peptides [104]. A cholesterol-binding domain within Aβ peptides was identified [100]. Very recently it was demonstrated that cholesterol, bound to lipid membranes, promoted Aβ aggregation by enhancing the primary nucleation rate by up to 20-fold [106]. This catalytic process might be the link between impaired cholesterol homeostatis and AD.

To summarize, the cholesterol-lipid raft/APP-Aβ interactions are very complex and need further studies. During aging, the cholesterol content of the cellular membranes is decreasing and that favors the translocation of APP and intracellular APP-processing to Aβ [107].

In the brain of AD patients, the microtubule-associated protein tau is found in various assemblies, including soluble oligomers and insoluble β-structured fibrils, the paired helical filaments (PHFs) [14]. Tau is an aggregation-prone protein and forms very easily β-structures. Interactions of tau in the suitable environment of membranes could provide oligomerization and aggregation into PHF-s [108]. Tau interacts with membranes and micelles and these interactions modulate the fibrillation pathway [109]. Promoting tau aggregation is a suicidal process for the membranes: fibrillization and PHF formation lead to disruption of membranes [32]. PHFs contain also cholesterol, PC, and sphingolipids [110]. Tau-membrane interactions and PHF formation may play a pathological role in AD.

4.5. Lipids as Biomarkers of AD

As lipid perturbations are involved in AD pathology, lipids are potential biomarkers for AD. Although the lipidome has unprecedented complexity, lipid changes could be used as prognostic-, susceptibility- and monitoring biomarkers in AD. As such, these markers may serve for identification of AD patients at risk for developing mild cognitive impairment (MCI) or transition from MCI state to AD. The early diagnosis and stratifying MCI and AD subjects to different cohorts would be crucial for patient recruitment. Several types of biomarkers for AD diagnosis (noninvasive imaging, cerebrospinal fluid biomarkers, genetic biomarkers, *etc.*) have been used, but they are time consuming, invasive, or expensive. Blood based biomarkers (proteins, lipids) have many advantages (blood extraction is easy, both time and cost efficiency; repeated sampling). Detecting brain-related alterations in blood lipid profiles may give biologically relevant peripheral signals of AD progression [111].

During the last seven years, a lot of studies were performed for finding reliable blood biomarkers using lipidomics techniques. An interesting trial measured the lipid levels of plasma of demented patients and identified an altered sphingolipidome using shotgun methods [112]. Another study investigated the level of plasma sphingomyelins and found association with the cognitive decline in AD [72]. Mapstone *et al.* described and validated a set of ten plasma metabolite-lipids that predicted the development of MCI or AD with over 90% accuracy [113]. This biomarker panel reflects cell membrane integrity and might be sensitive to early neurodegeneration of prodromal AD. The proposed panel of lipid metabolites may distinguish between subjects who would progress to a MCI/AD or maintain cognitive capacities within 2 to 3 years.

The change of plasma levels of long-chain PUFA (such as, DHA) for the diagnosis of brain atrophy and cognitive decline was also measured [114]. In another study, hexacosanoic acid (C26:0) was identified as a blood lipid marker of dementia [115]. It was also demonstrated that long chain cholesteryl esters in the plasma were associated with AD [116]. A comprehensive lipidome analysis resulted in a lipid panel consisting of a combination of 24 molecules that was used as biomarkers in AD with 70% accuracy [116]. Plasma sphingolipids could be diagnostic and/or prognostic biomarkers for AD [72]. Recent studies demonstrated that CSF-sphingolipids are elevated in AD patients and may indicate disease severity. Elevated blood ceramide levels hints to cognitive impairment and development of AD among cognitively normal individuals. A recent study analyzed plasma lipidome changes in individuals having an autosomal-dominant (PS1) AD-mutation. PPE 34:2 and PPE 36:4 correlated with CSF tau as well as with amyloid load [117]. Further experiments confirmed the disturbance of sphingolipid metabolism in early phase of AD [118] and proved the relation between brain and blood sphingolipids and AD pathology. According to Varma *et al.* sphingolipids may be used in the future for early detection of AD. Very recently Zarrouk *et al.* widely reviewed the lipid biomarkers of AD and found that the presence of very long chain fatty acids may indicate peroxisomal dysfunction in AD and suggest new targets in AD drug research [119].

Taken together, accurate blood-based lipid biomarkers could constitute simple AD-diagnostic tools in the future.

5. TOXIC EFFECT OF AB AGGREGATES ON SUBCELLULAR ORGANELLES

Aβ-membrane interactions may play an important role in neuronal damage in AD. Hence, Aβ-membrane interactions provide therapeutic targets [120]. Since large fibrillary amyloid aggregates bind to the cell surface, toxic Aβ assemblies induce morphological changes in mammal cells and affect neuronal cells elasticity [39].

In the pathogenesis of AD, mitochondria are central players. Mitochondrial dysfunction is an early feature of AD. Several evidence suggest that APP and Aβ accumulates in mitochondrial membranes causing structural and functional damage that prevents the normal function of neurons [121, 122]. Aβ oligomers induce a massive Ca^{2+}-influx into neurons and promote mitochondrial Ca^{2+}-overload [123]. Aβ induces mitochondrial permeability transition, the release of cytochrome-c, and apoptotic cell death. Increased spatial association of Aβ with mitochondria is linked with reduced mitochondrial respiration [124]. Both extra- and intracellular Aβ are able to transport into the mitochondria via a receptor-dependent pathway through the translocase of outer membrane (TOM40) machinery or through direct endoplasmic reticulum (ER)-mitochondria transfer [125, 126]. The mitotoxicity induced by Aβ is still not clear but includes numerous mechanisms. Mitochondrial Aβ decreases cytochrome oxidase activity [122] and interacts with mitochondrial matrix protein cyclophilin D [127, 128]. Aβ binds to the members of the dehydrogenase enzyme family. For example, binding of Aβ to alcohol dehydrogenase (ABAD) directly affects mitochondrial respiratory enzyme activity inhibiting complex IV and triggers mitochondrial membrane permeability by transition pore opening. The interaction between mitochondrial enzymes and Aβ was widely reviewed [129]. In summary, Aβ disrupts mitochondria as the energy powerhouse of neurons, decreases ATP generation, and increases mitochondrial ROS production [130]. Aβ damages the mitochondrial structure, increases the production of defective mitochondria, and decreases mitochondrial trafficking [121].

Beside mitochondria, the functions of the ER are damaged by Aβ. Disruption of ER architecture is deleterious for normal cell physiology. Chronic activation of the unfolded protein response (UPR) might lead to apoptotic cell death. Increased levels of intracellular Aβ induces mild ER-stress and UPR [131] and may trigger apoptosis [132], however, this process requires functional mitochondria owing to the ER-mitochondria crosstalk. Aβ-triggered ER stress promoted cholesterol synthesis and mitochondrial cholesterol influx in an AD mouse model [133]. It was observed that oligomeric Aβ42 decreased ER Ca^{2+}-levels resulting in intracellular Ca^{2+}-dyshomeostasis [134]. It was also found that low molecular weight Aβ (soluble oligomers) decreased the stability of the microtubular (MT) (tubulin-containing) structures that direct the movement of these organelles and as such disrupted the anchoring between ER and MT [135]. Also, Aβ caused neurovascular dysfunction by inducing ER-stress in brain endothelial cells [136]. Aβ-dependent dysregulation of cellular Ca^{2+} homeostasis is the result of activation of IP_3 receptor and Ca^{2+}-release from the ER [137].

6. OXIDATIVE STRESS, LIPID PEROXIDATION AND 4-HYDROXY-2-NONENAL IN AD. THE "CALPAIN-CATHEPSIN" HYPOTHESIS.

6.1. Protective Stress Signaling Pathways Activated by Electrophiles

As highly reactive by products of aerobic respiration, reactive oxygen species such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxide radicals (OH^-) can damage lipids, proteins, or DNA; a process called oxidative stress. However, the current oxidative stress paradigm needs reappraisal as the biological action of reactive species is not corroborated by experimental data [138]. Nowadays, these reactive species are also recognised as signaling mediators which are produced and inactivated in a regular manner in a process called redox signaling [139]. Hence, the "redox biology paradigm" was suggested in which antioxidants control cell signaling and metabolism [138]. The major physiological second messengers that modulate the pre-existing redox networks include nitric oxide, hydrogen peroxide, and electrophiles. Next to being abundant diet constituents, electrophilic lipids (HNE, oxononenal, acrolein, cyclopentenone-structured prostaglandins, and PC-derived aldehydes) are also produced through enzymatic and non-enzymatic lipid peroxidation. As discussed below, electrophile-modulated signaling pathways can be exploited for novel therapeutic interventions in major neurodegenerative disorders, including AD.

Under conditions of extensive formation of reactive oxygen and nitrogen species (ROS and RNS), unsaturated fatty acids undergo radical reactions resulting in a variety of biologically active electrophilic species. Ultimately, moderate exposure to these electrophilic products evokes protective cell signaling responses such as the Keap1-Nrf2 (Kelch-like ECH-associated protein – nuclear factor E2-related factor) pathway, the heat shock response pathway (HSR), and the UPR pathway (UPR, Fig. **4**).

The Keap1-Nrf2 pathway regulates cellular responses to oxidative and electrophilic stress [140-142]. Under basal conditions, the redox-sensitive Keap1 tethers Nrf2, resulting in its ubiquitin- and cullin-3-dependent proteasomal degradation. During electrophilic stress, Keap1 cannot longer assist in Nrf2 ubiquitination, which leads to Nrf2 stabilisation. Keap1 has a total of 27 thiol residues in human which are adducted in a reactive electrophile pressure-dependent pattern [143, 144]. Hence, the "cysteine code" postulates that each electrophile covalently modifies specific cysteines of a given group of proteins [145]. Target genes of Nrf2 include xenobiotic metabolizing enzymes (*e.g.* glutathione S-transferases and NAD(P)H:quinone oxidoreductase-1), antioxidant enzymes (*e.g.* haem oxygenase1), enzymes involved in glutathione metabolism (*e.g.* glutamate cysteine ligase), and recycling, and proteasomal and chaperone proteins [142, 146, 147].

In addition to the Keap1-Nrf2 pathway, electrophiles also activate the heat shock response (HSR) [148]. Although how precisely electrophiles induce HSR is yet undefined, both Hsp70 and Hsp90 are modified by HNE [149]. Most likely, this binding disrupts the pre-existing HSF1-Hsp70-Hsp90 complex, which represses HSF1 under normal conditions [150].

Interestingly, recently it was reported that Nrf2 is activated by heat shock through increased Hsp90 and Keap1 interaction and subsequent dissociation of the Cul3-Keap1-Nrf2 complex suggesting that the two pathways interact [151].

The UPR is also regulated by electrophiles [152]. UPR is induced through disturbances in ER proteostasis and modulates transcription and translation in order to re-establish ER

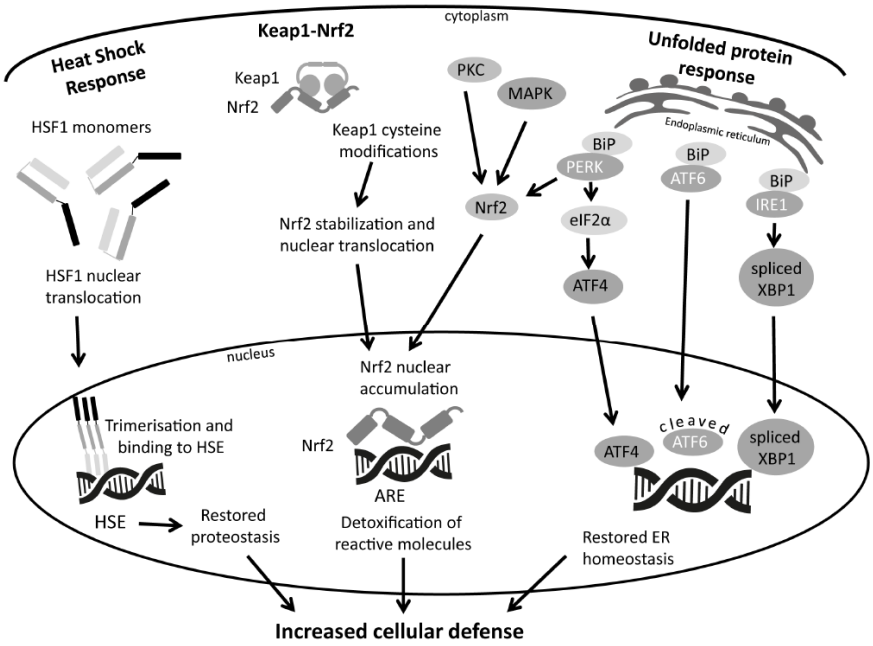

Fig. (4). Electrophile-induced stress signaling pathways. Heat shock response. Once activated, heat shock factors (HSFs) migrate to the nucleus, and bind as a trimeric complex to heat shock elements resulting in enhanced expression levels, of among others, HSPs. Keap1-Nrf2 pathway. During electrophile stress, Keap1 cannot deliver Nrf2 to the proteasome. Thus, Nrf2 migrates to the nucleus, where it binds to antioxidant response elements to drive expression of specific genes. Posttranslational modification of Nrf2 by PKC, PERK, or MAPK enables Nrf2 to migrate to the nucleus without the help of Keap1. Unfolded protein response (UPR) – deregulated ER proteostasis activates the different branches of the unfolded protein response. Ultimately, three different transcription factors are activated which drive the expression of unfolded protein response specific genes. (ARE: antioxidant responsive element)

homeostasis [153]. UPR consists of three distinct pathways depending on transmembrane signaling proteins: activating transcription factor-6 (ATF6), inositol requiring protein-1 (IRE1), and PERK. Additionally, the chaperone BiP can form complexes with the signaling proteins [153]. Electrophilic lipids including HNE, oxononenal, acrolein, cyclopentenone prostaglandins, and phosphatidylcholine-derived aldehydes POVPC and PEIPC can all activate UPR [152, 154]. Although, it is still unclear how these electrophilic lipids regulate UPR, most likely they function through ER-resident chaperones [154-157]. Thus, modification of ER-resident chaperones disturbs proteostasis and activates UPR [158].

Interestingly, ER stress activates Nrf2 via direct PERK-dependent phosphorylation of Nrf2 [159] whereas ATF4 is a Nrf2 target gene [160], suggesting overlap between the Keap1-Nrf2 and UPR pathways.

6.2. The Calpain Cathepsin Hypothesis and the "Janus-faced" HNE

Autophagy is essential to maintain cellular homeostasis [161] and can be classified in three types [162]: (1) macroautophagy-cytosolic organelles and/or macromolecules are engulfed by the autophagosome and delivered to the lysosome for degradation, (2) chaperone-mediated autophagy – damaged, and aged proteins are recognised by Hsp70 and delivered to the lysosome through association with the lysosome-associated membrane protein type 2A (Lamp-2A), and (3) microautophagy – direct engulfment of cytoplasmic cargo by the lysosomal membrane [163-165].

Internal lysosomal membranes are characterized by bis(monoacylglycero) phosphate (BMP), an abundant lysosome-specific anionic phospholipid [166, 167]. BMP associates with acid sphingomyelinase and drives hydrolysis of sphingomyelin to ceramide [168-170]. Subsequently, ceramide stabilizes lipid phases, [171, 172] and prevents lysosomal membrane rupture [173-175].

In chaperone-mediated autophagy, chaperone-bound misfolded proteins associate with Lamp-2A to form an oligomeric Lamp-2A translocation complex [176]. Thus, chaperone-mediated autophagy substrates transfer to the lysosomal lumen where they unfold and dissociate from the chaperones. Meanwhile, lysosomal-resident Hsp90 and he cytosolic pool of glial acidic fibrillary protein stabilizes Lamp-2A [176]. Cytosolic proteins and organelles destined for autophagic degradation are delivered to lysosomes in complex with Hsp70.

This complex binds to Lamp-2A [177], upon which the substrate proteins are transferred to the lysosome for degradation with the help of luminal Hsp70 [178]. Importantly, during this process, Hsp70 is not only crucial as a molecular chaperone but also stabilizes the outer lysosomal membrane [173, 179-181]. Several studies suggest that oxidative damage, especially by linoleic- or arachidonic acid-derived HNE occurs in Alzheimer's disease patients. For instance, elevated levels of HNE were measured in the brain tissue [182], ventricular fluid [183], the amyloid component of senile plaques [184], and in the plasma of the Alzheimer patients [185]. In response to HNE, Hsp70 gets carbonylated at Arg469 [186], see (Fig. **5A**) (see references in [187]).

The role of calpains in cell death has been demonstrated. Calpains – cysteine proteases abundantly expressed in neurons and implicated in multiple neurological functions – are involved in APP processing, neurofibril increase, and neuronal death in the anterior frontal lobes of the AD brain [188]. Among others, lysosomal membrane-associated Hsp70 was identified as a specific calpain substrate [189]. Cleavage of Hsp70 through calpain in parallel with HNE was demonstrated in the murine photoreceptor methylnitrosourea-induced cell death model [190]. Hsp70 causes the formation of a ceramide layer on the lysosomal membrane through activation of ASM. Thus, cleavage of Hsp70 through calpain deregulates ASM resulting in enhanced levels of sphingomyelin and decreased levels of ceramide at the lysosomal membrane [191, 192]. Ultimately, this results in lysosomal disruption and/or rupture in AD neurons and the release of lysosomal, hydrolytic cathepsin enzymes damage cellular proteins as well as the outer lysosomal membrane. In parallel, activated phospholipases degrade multiple types of cellular membranes. In addition, mitochondria are disrupted what results in the release of pro-apoptotic factors while hampering of the electron-transporting complexes causes H_2O_2 production [193]. Taken together, the 'calpain–cathepsin hypothesis' postulates that calpain-mediated disturbance of lysosomal membranes and subsequent cytoplasmic cathepsin release could represent a central cascade for degenerative neuronal cell death [187, 191, 194, 195].

However, HNE is a typical Janus-faced molecule, with both neurotoxic and neuroprotective effect. Neuroprotection is performed via Daxx (Death association protein 6), a transcription repressor molecule (Fig. **5B**). Daxx can negatively regulate the expression of stress responsive genes through an inhibitory interaction with HSF1 [196]. HNE-induced modification of Daxx results in its translocation to the cytoplasm [197-199]. As such, HNE-induced translocation of Daxx from the nucleus to the cytoplasm releases HSF1 and allows it to bind to its DNA recognition elements to drive expression of Hsps [198]. Thus, a Janus-faced model of HNE is proposed in which, at the one hand, HNE results in carbonylation-induced cleavage of Hsp70 while, at the other hand, HNE induces Hsp expression through nuclear export of Daxx and subsequent HSF1 activation (Fig. **5**).

7. PLASMA MEMBRANES AS STRESS SENSORS. THE THEORETICAL BACKGROUND OF MEMBRANE-LIPID THERAPY IN AD

Cellular membranes are dynamic lipid-protein structures in which lipid rafts provide an interface for protein-lipid interactions. The precise and timely localisation of specific signaling proteins into lipid rafts is governed by protein-lipid interactions. Specific membrane lipid-derived secondary messengers have pivotal roles in signaling cascades while rafts regulate the triggering of specific signaling pathways. One such membrane-originating signaling cascade is the stress response of which the fundamental principles will be described in this section. Finally, as changes in lipid composition are capable to alter signaling cascades that are related with neurodegenerative disorders including AD, membrane lipid therapy (MLT) will be discussed at the end of the chapter as a potential therapeutic strategy.

7.1. Membrane Sensor Hypothesis

The key role of heat shock proteins (Hsps) in cellular quality control and disposal of toxic proteins in neuroprotection is recently reviewed [162, 200]. As chaperone mole-

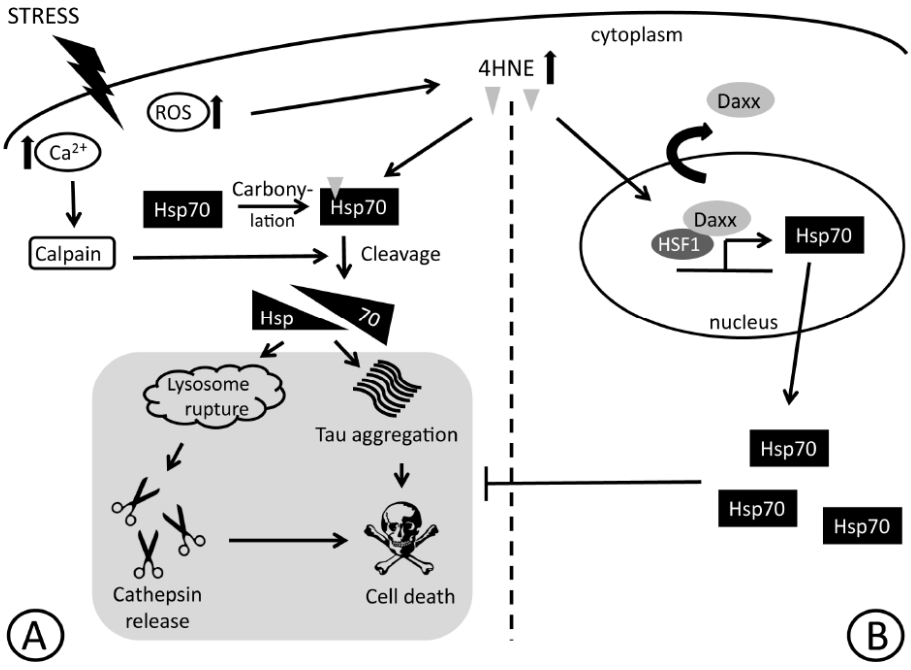

Fig. (5). Janus-faced character of HNE. **A**. Stress-induced elevation in ROS causes in lipid peroxidation and HNE formation. HNE causes carbonylation of Hsp70 which results in calpain-induced Hsp70 cleavage. This results in tau aggregation and cathepsin release through lysosomal rupture, both of which lead to cellular apoptosis. **B**. HNE induces nuclear export of the HSF1 inhibitor Daxx resulting in HSF1 activation. Enhanced Hsp70 expression levels might counteract lysosomal rupture and tau aggregation. (Daxx: Death associated protein 6)

cules, Hsps regulate correct folding of newly translated proteins and are responsible for the degradation of unneeded proteins to preserve normal cellular protein homeostasis ("proteostasis") [201]. By protecting and stabilizing membranes [202, 203] as well as preventing apoptosis through inhibition of stress kinases [204] or the caspase cascade [205], Hsps maintain cell integrity. However, during cellular stress and in certain pathological conditions (typically in neurodegeneration), increased chaperone capacity to avoid conformational changes or aggregation of misfolded proteins.

Originally, the HSR was associated with protein denaturation. However, in many circumstances, induction of Hsps happens without the occurrence of protein denaturation [206]. Hence, the "Membrane Sensor Hypothesis" predicts that Hsp levels can change as a result of temperature-induced plasma membrane alterations [207, 208], even during a fever-like mild heat shock [209]. Thus, heat stress is coupled with rapid changes in lipid metabolism and structural rearrangements of the plasma membrane which ultimately play a regulatory role in Hsp expression. The operation of the "Membrane Sensor Hypothesis" is apparently universal and verified from prokaryotes and plants to yeast and mammalian cells [210, 211]. In yeast, elevated temperatures resulted in enhanced sphingolipid metabolism and accumulated phyto- and dihydroceramide [212]. In parallel, complex sphingolipids are hydrolysed what results in stress-induced ceramide formation [212].

In mammalian cells, heat induces immediate membrane hyperfluidization which is quickly followed by changes in lipid composition [213] and hence membrane structural properties [40, 214-216]. As such, heat stress results in an immediate and specific reorganization of cholesterol-rich lipid rafts and an enhanced packing density of the lipids residing in the plasma membrane [40, 214, 216]. Meanwhile, the level of saturated lipid species enhances through the activity of phospholipase PLA2 and PLC. The activation of the PLC–diacylglycerol (DAG) lipase–monoacylglycerol (MAG) lipase pathway produces multiple lipid mediators among which the strong HS mediator ARA [217].

7.2. The Complex Signaling Network of Heat Stress and the Membrane Lipid Therapy

The immediate activation of plasma membrane localised signaling cascades upon heat stress exposure was recently reviewed (see [207] and references therein). In this paragraph, only a brief summary will be given. Enhanced plasma membrane fluidity activates calcium channels, such as TRPV channels, what results in a transient increase in the cytosolic Ca^{2+} concentration which in turn activates calmodulin kinase II (CaMKII), and cytosolic phospholipase A2 (cPLA2). In parallel, disturbance of membrane lipid environment and lipid rafts causes growth factor receptor activation in the absence of its ligand and triggers small GTPases (Rac1) resulting in active PLC and MAPK. PLC activation results in a rapid fall in PIP2 and PIP levels and a consequent IP3 and DAG generation. Generation of DAG results in activation of PKC and the formation of ARA through the DAG- MAG lipase pathway. PKC activation result in activation of cPLA2. On its turn, PLA2 contributes to ARA production. ARA can be further metabolized to eicosanoids – well known HSR inducers. In parallel, GFR kinases activate PI3K which convert PIP2 to phosphatidylinositol-3,4,5-triphosphate (PIP3) which trigger AKT and Rac1. AKT inhibits the glycogen synthase kinase-3 (GSK3) and activates mTOR, ultimately resulting in activation of HSF1. In parallel, Rac1 activation has a regulatory role in the Hsp response. In addition, upon stress, sphingomyelin is hydrolysed to ceramide or sphingosylphosphorylcholine by acid sphingomyelinase or sphingomyelin deacylase, respectively. Ceramide can be deacylated to sphingosine by ceramidase or glycosylated to glucosylceramide by glucosylceramide synthase. Sphingosine is subsequently phosphorylated to spingosine-1-phosphate by sphingosine kinase 1 which ultimately triggers expression of Hsp through the activity of p38 MAPK and PI3K. The glucose group of glucosylceramide can be switched to cholesterol by glucosyltransferase to form cholesteryl glucoside which activates Hsp expression.

Ceramide-enriched membrane microdomains are formed through competition of enhanced levels of ceramide with existing pools of cholesterol for raft localisation. Sphingosylphosphorylcholine triggers p38 MAPK signaling resulting in enhanced Hsp expression. Ultimately, an altered lipid composition of the rafts modulates its signaling characteristics through changes of the raft proteome as such allowing the cell to cope with the implied stress [218].

Changes in lipid composition are capable to alter signaling cascades that are related with pathologies including diabetes, cancer, cardiovascular pathologies, or neurodegenerative disorders. This concept was first introduced by Escriba and named "Membrane lipid therapy" [219]. MLT aims to modify the activity of pathology-specific signaling pathways through the pharmaceutical use of molecules able to alter the membrane lipid environment of lipid raft structures [208, 218]. The MLT concept postulates that specific membrane lipids can be modified to change the structure or composition of the plasma membrane [208]. As such, the localisation and/or activity of specific proteins in lipid rafts could be affected and ultimately modulate malfunctioning lipid signaling cascades. Proteins localise to lipid rafts through specific protein-lipid interactions. This is influenced by specific lipid classes (PS, PI, PE, or DAG) or membrane lipid structures (lamellar-prone or HII-prone bilayers) which as such facilitate timely protein–protein interactions and their subsequent signaling outputs [220]. On the other hand, the interaction of proteins belonging to signaling cascades is determined by the membrane lipid structure. For example, environmental changes are sensed by lipid rafts or caveolae which subsequently regulate the stress response through their specific occurrence and distribution pattern [221, 222]. Otherwise, the pre-existing structure and order of particular membrane domains can be modulated through interaction with specific Hsps [202]. Thus, the heat-induced membrane lipid disorganization is antagonized by this feed-back loop which preserves, at least temporarily, the structural and functional integrity of the membrane during stressful conditions. As such, specific interactions between Hsps and specific lipids allow Hsps to be targeted to distinct membrane subdomains (rafts) known to be pivotal in multiple signaling pathways [208]. In fact, a "unifying theory" focusing on microdomains as vital players in a new model of gene regulation can be postulated

in which membrane physical state and microdomain structure are related to the regulation of Hsp expression, as well as to the feedback of specific Hsps in preserving/restoring the structural and/or functional properties of the membrane [206]. Thus, by affecting the membrane physical state, membrane-localised signaling pathways can be controlled with obvious widespread consequences in health and disease.

A novel group of durgs, hydroximic acid derivatives intercalate with biological membranes. These compounds are able to reduce the molecular order of specific membrane domains and to correct dysregulated expression of Hsps at the same time [215, 223]. These drugs have been used for the treatment of neurological and neuromuscular diseases, including amyotrophic lateral sclerosis [224], Huntington's disease [225], and muscular dystrophy [226].

8. THERAPIES INVOLVING LIPIDS IN PREVENTION AND TREATMENT OF AD

8.1. Membrane Lipid Factors in AD

Pharmaceutical or nutraceutical interventions are able to modulate the membrane lipid composition and would allow normalizing specific signals which were altered under certain (pathological) conditions. Natural or synthetic lipids could target the lipid composition of the overall plasma membrane, of the residing microdomains, or of the cellular organelles [227-229]. In fact, synthetic fatty acids designed to modulate the organization of membrane microdomains similar to their natural counterparts [230] were able to modulate interactions between specific proteins in membrane microdomains [231].

Dietary lipids could be directly incorporated into cell membranes where they regulate the activity of membrane proteins. As heat-induced ceramide production correlates with specific Hsp induction in NIH WT-3T3 cells [232], the formation of ceramide-rafts may control stress signals across the plasma membrane. Both the exogenous ceramide analogue C2-Cer and the increase of the endogenous intracellular ceramide induce the sHsp ab-crystallin, but not the structurally related Hsp27 [233]. In isolated rat cerebral arteries, sphingosylphosphorylcholine activates Hsp27 via the p38 MAPK pathway [234]. In cell cultures, sphingosine-1-phosphate induces Hsp27 release via the p38 MAPK and PI3K/Akt pathways [235, 236]. Exogenous cholesteryl glucoside, which under HS is derived from cholesterol, rapidly activates HSF1 and initiates Hsp70 production in fibroblasts [237, 238]. These findings demonstrate the key role of lipids in fine tuning the expression of Hsp chaperones. Application of dietary or nutraceutical lipids may be beneficial in the treatment of several NDDs [222].

A recent new principle in biology, the xenohormesis postulates that environmentally stressed plants produce bioactive compounds and these substances can increase stress resistance and survival benefits to their consumers [239]. For example, plants exposed to cold shock synthesize higher amount of unsaturated fatty acids for increasing their membrane fluidity by membrane stabilization [240]. As the hyperfluidization of mammalian cell membranes acts as a signal to initiate the heat shock protein response [216, 241], animal consumption of these less saturated fats lowers the animal's threshold for triggering the stress response and is associated with a less disease-prone state. Evidently, the positive impact of "healthy" plant and animal oils (*e.g.*, olive oil and omega-3 fatty acids) on our well-being is well known [242, 243]. As another example, ingested of stress-induced plant-derived phenolic compounds – *e.g.* flavonoids (including rutin, anthocyanidins) and nonflavonoids (including resveratrol, curcumin) – activate the mammalian stress response, have antioxidant and anti-inflammatory effects, and might be used in the therapy of NDDs [244-247].

8.2. Lipid Dietary Therapies in AD

From the early 1980s onwards, scientists began to study the role of nutrition in cognitive processes. For example, several studies (such as the Rotterdam study) demonstrated that the consumption of ω-3 fatty acid-rich fish enhanced cognitive performances in the elderly whereas the excess linoleic acid had the opposite effects [248]. In addition, several epidemiological studies were performed in Norway, France, China, and the United States. In the Chinese- as well as in the Bergen-Oslo study it was found that the subjects consuming an average of more than 10g/day fish or seafood had significantly higher scores in psychometrics tests [249]. The Chinese study gave very similar results [250]. The results of the French study supported the hypothesis of a beneficial effect of foods rich in ω-3 fatty acids in the prevention of cognitive decline [251]. In the American study the authors observed better cognition capacities, mainly in verbal memory, in people consuming one or more dark-meat fish (tuna, salmon, mackerel, *etc.*) [252].

A Swedish study from the Uppsala research group [253] found positive correlations between EPA and DHA dietary intake, overall cognition performances, and the grey matter volume measured by MRI.

Many studies indicated that the absolute amount of ω-3 fatty acids is in fact a less important marker than the ω-6 to ω-3 ratio. The "Trois Cités" (Bordeaux, Dijon, Montpellier) study showed that a high consumption of ω-6 fatty acid-rich oils increased the risk of dementia and even AD [254]. These results might be explained by the well-known metabolic competition between these two fatty acid series. Despite certain limitations, the cited research results suggest that a dose ranging from 1 to 2 gram of DHA per day can decrease cognitive decline in healthy subjects and also in mild forms of dementia and prodromal AD [255]; but another study pointed out that this kind of slowdown effect failed when applied to elderly subjects [256].

The loss of memory and learning that accompanies Alzheimer's disease correlates with a decline in DHA - the most abundant FA in neuronal membranes in the cerebral cortex grey matter [257] and the dietary intake of ω-3PUFA decreased the risk of AD [258]. A 4-months treatment with the synthetic 2-hydroxy-DHA (LP226A1, Lipopharma) in a mouse model of AD (5XFAD mice) resulted in increased neurogenesis and normal cognitive scores in a behavior test [229].

In some MCI patients, a progressive worsening of their cognitive functions is seen which might progress to AD. The DHA content of the AD brain is reduced [259], (Section

Table 2. Therapies involving lipids in prevention and treatment of AD.

	Alleviating effects	References
Membrane lipid therapy		
Phosphatidylserine	-Improved cognitive functions in AD patients	[228]
2-hydroxydocosahexaenoic acid	- Decreased amyloid-β (Aβ) accumulation and full recovery of cognitive scores in mouse model of AD (5xFAD)	[229]
Dietary interventions		
Classical ketogenic diets	- Decreased level of brain Aβ40 and Aβ42 in a transgenic mouse model of AD (APP/V717I)	[266]
	- Improved cognitive functions in epileptic children	[267, 268]
Medium chain triglycerides	- Improved cognitive performance in memory-impaired subjects	[269, 270]
	- Cognitive improvement in a patient with younger onset sporadic AD	[271]
	- A trend towards a decrease in level of Aβ and decrease in APP in the brain of aged dogs	[272]
Ketone esters	- Improvement in learning and memory tests and decreased Aβ and hyperphosphorylated tau deposition in a transgenic model of AD (3xTgAD mice)	[273]
	- Improved cognitive performance in a patient with younger onset sporadic AD	[271]

4.1.2). Interestingly, a low level of EPA (but not DHA) in the erythrocytes proved to be a good indicator of decreasing cognitive performance [260]. In fact, supplementation with EPA and DHA lowers amyloid plaque formation by reducing Aβ accumulation via the glymphatic system [261]. However, the EPA- and DHA-derived lipid mediators oxylipins (Section 4.1.2) might have adverse effects by increasing neuroinflammation [262] which is counteracted by the anti-inflammatory effects of DHA thus improving neuronal survival. Seventeen of the 19 reliable AD epidemiological studies published between 1997 and 2008, reported that the risk of AD increased when the nutritional intake of ω-3 fatty acids decreased [263]. However, the achieved effects of PUFAs treatment were highly dependent on the state of disease development and most effective in the early stages of AD. Thus, as DHA is able to slow several deleterious molecular mechanisms of AD, an early nutritional intervention (*e.g.* administration of DHA and antioxidants) is necessary from the early onset of AD.

Several recent trials demonstrated promising results. For example, in the LipiDiDiet European project, daily intake of a dietary supplement (Nutritia Souvenaid R) providing 1200 mg DHA and 300 mg EPA, vitamins (E, B6, B12), trace elements (selenium), and various metabolites (uridine, choline) showed beneficial effects on memory and behaviour [264] and in neuronal network organization [265]. Currently, at least 15 dietary projects on ω-3 FA supplementation are scheduled or to be completed in different research centres globally. However, as DHA was most effective in inhibiting amyloid plaque generation and slowing down the cognitive decline in patients lacking the ApoE4 gene, ApoE polymorphisms might have to be considered in future trials [263].

8.3. Classical Ketogenic Diet and Exogenous Ketone Supplements in AD Therapy

Ketone bodies such as D-beta-hydroxybutyrate (R-3-hydroxybutyrate/βHB), acetoacetate and acetone are mainly synthetized in the liver from fatty acids under normal physiological conditions and particularly during fasting, starvation and neonatal development [274, 275]. Ketone bodies can enter into neurons through monocarboxylic transporters, convert to acetyl CoA in mitochondria and enter to Krebs cycle providing a source of energy for the central nervous system. Moreover, ketone bodies not only improve mitochondrial respiration but also decrease ROS formation [276] and direct application of βHB evoked protective effects against Aβ-induced cell death in cultured neurons [277]. Also, βHB can stimulate chaperone-mediated autophagy, in which βHB-evoked processes may eliminate accumulated axonal proteins such as Aβ in AD [278, 279]. In addition, ketone bodies blocked the entry of Aβ into neurons/neuronal mitochondria and improved learning and memory in a mouse model of AD [280]. Thus, using dietary interventions such as classical ketogenic diets (KDs) or exogenous ketone supplements (normal food plus ketone supplements such as ketone esters and/or medium chain triglycerides), which are able to evoke and maintain a rapid and safe mild ketonemia/ketosis (therapeutic hyperketonemia) [281-283] may have therapeutic effect in AD and may decrease the risk of this disease.

It has been revealed that classical KDs (low in carbohydrate and adequate in protein content and high in fat content; primary fat source is long-chain triglycerides) improve mitochondrial functions, promote mitochondrial biogenesis and ATP synthesis and decrease ROS formation by activation of mitochondrial uncoupling proteins [284, 285] suggesting a

potential therapeutic benefit of KD in AD. Indeed, a KD decreased the level of brain Aβ40 and Aβ42 level in a transgenic mouse model of AD (APP/V717I, expressing APP gene containing the "London" APP mutation) [266] although improvement in novel object recognition test could not be demonstrated. Moreover, KD evoked improvement in cognitive functions in epileptic children [267, 268] whereas it impaired learning and memory in rats [286].

It has been demonstrated that administration of medium chain triglyceride, *e.g.* AC-1202 significantly improved cognitive performance in memory-impaired subjects [269, 270]. In a patient with younger onset sporadic AD, a medium chain triglyceride/medium-chain fatty acid mixture was shown to evoke cognitive improvement [271]. Nevertheless, a medium chain triglyceride rich diet did not rescue memory deficits and was not able to evoke changes in Aβ as well as tau deposition in two transgenic mouse models (amyloid-depositing mice APP/PS1 and tau depositing mice Tg4510) of AD [287] suggesting that this diet has minimal impact on these types of AD models. In contrast, medium chain triglyceride administration not only improved mitochondrial function but also showed a trend towards a decrease in level of Aβ and decrease in APP in the brain of aged dogs (a natural model of amyloidosis) [272]. In a transgenic model of AD (3xTgAD mice) it was demonstrated that a diet containing ketone ester evoked improvement in learning and memory tests and decreased Aβ and hyperphosphorylated tau deposition in the cognition-relevant brain areas such as hippocampus and cortex [273]. Moreover, ketone ester improved, among others, cognitive performance in a patient with younger onset sporadic AD [271]. It was hypothesized that before symptoms of AD develop, ketone supplements-evoked mild ketonemia may decrease the risk of further metabolic impairment and appearance of symptoms of AD such as cognitive decline [288].

CONCLUSION AND FUTURE PERSPECTIVES

In summary, more and more evidence hint to a strong relationship between brain lipid dyshomeostasis and AD. As most of the activity of the cells occurs within and around biomembranes, alterations in membrane lipid composition evidently play an important role in pathophysiological processes. In AD, many studies indicate a bidirectional link between APP and lipids. The APP processing and formation of Aβ peptides highly correlate with the composition of lipid rafts – formation of toxic Aβ-assemblies and tau-aggregates occurs inside lipid rafts through interaction of the initial non-toxic proteins with cholesterol and the ganglioside GM1 – whereas APP-derived cleavage peptides (Aβ, AICD) regulate lipid metabolism.

Intracellular Aβ oligomers may interact with the membranes of subcellular organs and deteriorate their functions. Moderate exposure to electrophilic lipids (*e.g.* HNE) also evokes protective stress signaling responses (*e.g.* Keap1-Nrf2) pathway) and increases cellular defense. HNE is a typical Janus-faced molecule and may trigger both neuroprotective process (via Daxx) and cell death (via lysosomal rupture). Heat stress may induce specific membrane remodeling, enhanced plasma membrane fluidity and triggers a complex signaling network. Specific membrane lipids and lipid nanostructures participate in cell signaling that are associated with neurodegenerative disorders. This is the base of membrane lipid therapy. Dietary factors may have a crucial role in the prevention and early treatment of AD, a lot of new trials are yet running. Preliminary results show the importance of ApoE4 protein and thus the Apoε4 gene in the result of dietary intervention. The ketonic diet that enhances the function of mitochondria also may be effective in AD prevention.

Although many relations between lipids and AD have been discovered, several questions remained unanswered. The detailed link between cholesterol metabolism and level as well as the initiation of AD process might be fully established. In the nearby future, blood-based biomarkers could constitute simple AD-diagnostic tools. The question whether specific lipid signatures predispose for AD or, on the contrary, protect against AD, will be answered. Lipid biomarkers will help in the identification of new targets, more effective drugs and new treatments.

ABBREVIATIONS

Aβ	=	Beta amyloid
AD	=	Alzheimer's disease
AFM	=	Atomic force microscopy
AICD	=	Intracellular APP-domain
Akt	=	(Protein Kinase B)
APP	=	β-Amyloid precursor protein
ARA	=	Arachidonic acid
ARE	=	Antioxidant responsible element
ASM	=	Acid sphingomyelinase
A2 (cPLA2)	=	Cytosolic phospholipase
BACE	=	β-secretase
βHB	=	D-beta-hydroxybutyrate (R-3-hydroxybutyrate)
BBB	=	Blood-brain barrier
CNS	=	Central nervous system
CD	=	Circular dichroism
CSF	=	Cerebrospinal fluid
DAG	=	Diacylglycerol
DHA	=	Docosahexaenoic acid
EPA	=	Eicosapentaenoic acid
ER	=	Endoplasmic reticulum
FA	=	Fatty acid
GPs	=	Glycerophospholipids
GWAS	=	Genome-wide association study
HNE	=	4-Hydoxy-2-nonenal
HSF	=	Heat shock factor
HSP	=	Heat shock protein
HSR	=	Heat shock response

IP3	=	Inositol 1,4,5-trisphosphate
IP3R	=	Inositol triphosphate receptor
KD	=	Ketogenic diet
MAG	=	Monoacyl glycerol
MAM	=	Mitochondria associated membrane
MAPK or MAP kinase	=	Mitogen-activated protein kinase
MD	=	Molecular dynamics
MLT	=	Membrane lipid therapy
mTOR	=	Mammalian target of rapamycin
MS	=	Mass spectrometry
MT	=	Microtubular
NDDs	=	Neurodegenerative diseases
p38 MAPK	=	P38 mitogen-activated protein kinases
PC	=	Phosphatidyl choline
PE	=	Phosphatidyl ethanolamine
PHF	=	Paired helical filament
PI	=	Phosphatidyl inositol
PIP	=	Phosphatidylinositol phosphate
PIP2	=	Phosphatidylinositol 4,5-bisphosphate
PIP3	=	Phosphatidylinositol trisphosphate
PI3K	=	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PKC	=	Protein kinase C
PLA2	=	Phospholipases A2
PLC	=	Phospholipase C
PLPP	=	Phospholipid phosphatases
PPE	=	Ethanolamine plasmalogene
PS	=	Phosphatidyl serine
PUFA	=	Poly unsaturated fatty acid
ROS	=	Reactive oxygen species
TEM	=	Transmission electron microscopy
UPR	=	Unfolded protein response

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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