REVIEW

Lipidomics of Alzheimer's disease: current status

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Abstract

Alzheimer's disease (AD) is a cognitive disorder with a number of complex neuropathologies, including, but not limited to, neurofibrillary tangles, neuritic plaques, neuronal shrinkage, hypomyelination, neuroinflammation and cholinergic dysfunction. The role of underlying pathological processes in the evolution of the cholinergic deficit responsible for cognitive decline has not been elucidated. Furthermore, generation of testable hypotheses for defining points of pharmacological intervention in AD are complicated by the large scale occurrence of older individuals dying with no cognitive impairment despite having a high burden of AD pathology (plaques and tangles). To further complicate these research challenges, there is no animal model that reproduces the combined hallmark neuropathologies of AD. These research limitations have stimulated the application of 'omics' technologies in AD research with the goals of defining biologic markers of disease and disease progression and uncovering potential points of pharmacological intervention for the design of AD therapeutics. In the case of sporadic AD, the dominant form of dementia, genomics has revealed that the ε4 allele of apolipoprotein E, a lipid transport/chaperone protein, is a susceptibility factor. This seminal observation points to the importance of lipid dynamics as an area of investigation in AD. In this regard, lipidomics studies have demonstrated that there are major deficits in brain structural glycerophospholipids and sphingolipids, as well as alterations in metabolites of these complex structural lipids, which act as signaling molecules. Peroxisomal dysfunction appears to be a key component of the changes in glycerophospholipid deficits. In this review, lipid alterations and their potential roles in the pathophysiology of AD are discussed.

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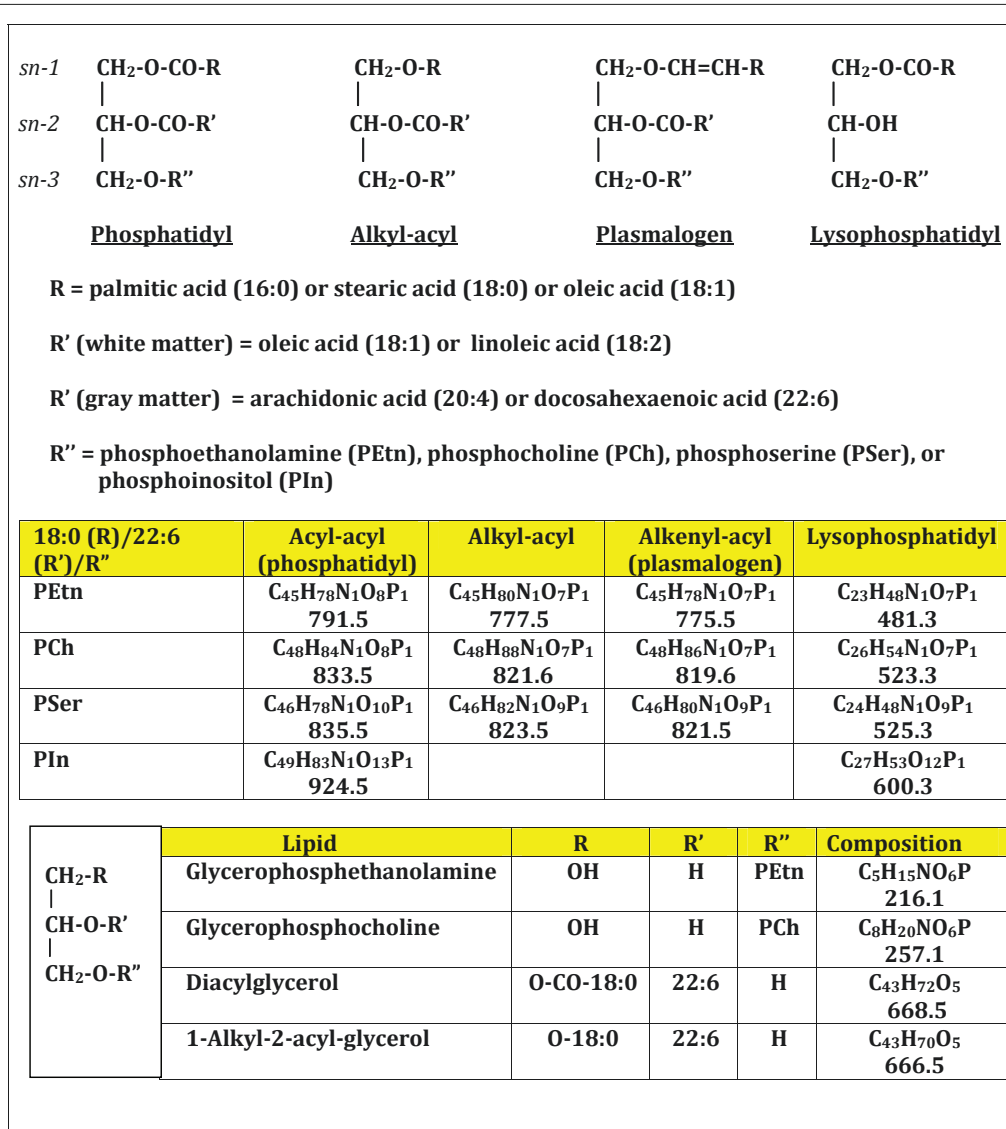
Introduction

There is no animal model of Alzheimer's disease (AD) that possesses the hallmark features of the disease: neurofibrillary tangles, neuritic plaques, neuroinflammation, neuronal shrinkage, hypomyelination and cholinergic dysfunction. This limitation to current AD research highlights the critical nature of the 'omics' technologies as tools to study AD patients and AD autopsy tissues. This review will focus on lipidomics findings in AD. The application of lipidomics to AD research is logical in that the brain is the most lipid-rich organ in the human body. In addition, genomics studies have consistently demonstrated that the ε4 allele of apolipoprotein E (ApoE), a lipid transport/chaperone protein, is a susceptibility, but not obligatory, factor for late onset (sporadic) AD. Furthermore, the severe cerebral macrostructural atrophy that is a hallmark feature of AD must involve the loss of structural lipids. Lipidomics has clearly shown that two major structural lipid classes are decreased in AD. These are the glycerophospholipids and sphingolipids. The current status of lipidomics findings and the potential roles of these lipid deficits in the pathophysiology of AD will be discussed. This review complements recent reviews of lipidomics in AD [1-3].

Glycerophospholipids

Ethanolamine glycerophospholipids

Early reports [4] of decreased phospholipid content in AD white matter were soon followed by reports of decreased levels of the phospholipid precursor ethanolamine in AD brain [5,6], cerebrospinal fluid (CSF) [7] and plasma [7] and increased brain levels of the degradation product glycerophosphoethanolamine [6]. The first descriptions of decrements in ethanolamine plasmalogens (PlsEtns), relative to phosphatidylethanolamines in AD brain, were published in 1995 [8]. Plasmalogens are a subclass of glycerophospholipids that possess a vinyl ether fatty alcohol substituent at sn-1 of the glycerol backbone (Figure 1). The ether linkage at sn-1 is achieved by addition of a fatty alcohol to the glycerol backbone and is conducted solely in peroxisomes (Figure 2). Subsequent desaturation to form the vinyl ether linkage takes place in the endoplasmic reticulum (Figure 2). Decrements in PlsEtns were shown to be disease specific since they were not measured in Huntington's caudate nucleus or

sn-1 CH₂-O-CO-R	CH₂-O-R	CH₂-O-CH=CH-R	CH₂-O-CO-R
sn-2 CH-O-CO-R'	CH-O-CO-R'	CH-O-CO-R'	CH-OH
sn-3 CH₂-O-R''	CH₂-O-R''	CH₂-O-R''	CH₂-O-R''
Phosphatidyl	**Alkyl-acyl**	**Plasmalogen**	**Lysophosphatidyl**

R = palmitic acid (16:0) or stearic acid (18:0) or oleic acid (18:1)

R' (white matter) = oleic acid (18:1) or linoleic acid (18:2)

R' (gray matter) = arachidonic acid (20:4) or docosahexaenoic acid (22:6)

R'' = phosphoethanolamine (PEtn), phosphocholine (PCh), phosphoserine (PSer), or phosphoinositol (PIn)

18:0 (R)/22:6 (R')/R''	Acyl-acyl (phosphatidyl)	Alkyl-acyl	Alkenyl-acyl (plasmalogen)	Lysophosphatidyl
PEtn	$C_{45}H_{78}N_1O_8P_1$ 791.5	$C_{45}H_{80}N_1O_7P_1$ 777.5	$C_{45}H_{78}N_1O_7P_1$ 775.5	$C_{23}H_{48}N_1O_7P_1$ 481.3
PCh	$C_{48}H_{84}N_1O_8P_1$ 833.5	$C_{48}H_{88}N_1O_7P_1$ 821.6	$C_{48}H_{86}N_1O_7P_1$ 819.6	$C_{26}H_{54}N_1O_7P_1$ 523.3
PSer	$C_{46}H_{78}N_1O_{10}P_1$ 835.5	$C_{46}H_{82}N_1O_9P_1$ 823.5	$C_{46}H_{80}N_1O_9P_1$ 821.5	$C_{24}H_{48}N_1O_9P_1$ 525.3
PIn	$C_{49}H_{83}N_1O_{13}P_1$ 924.5			$C_{27}H_{53}O_{12}P_1$ 600.3

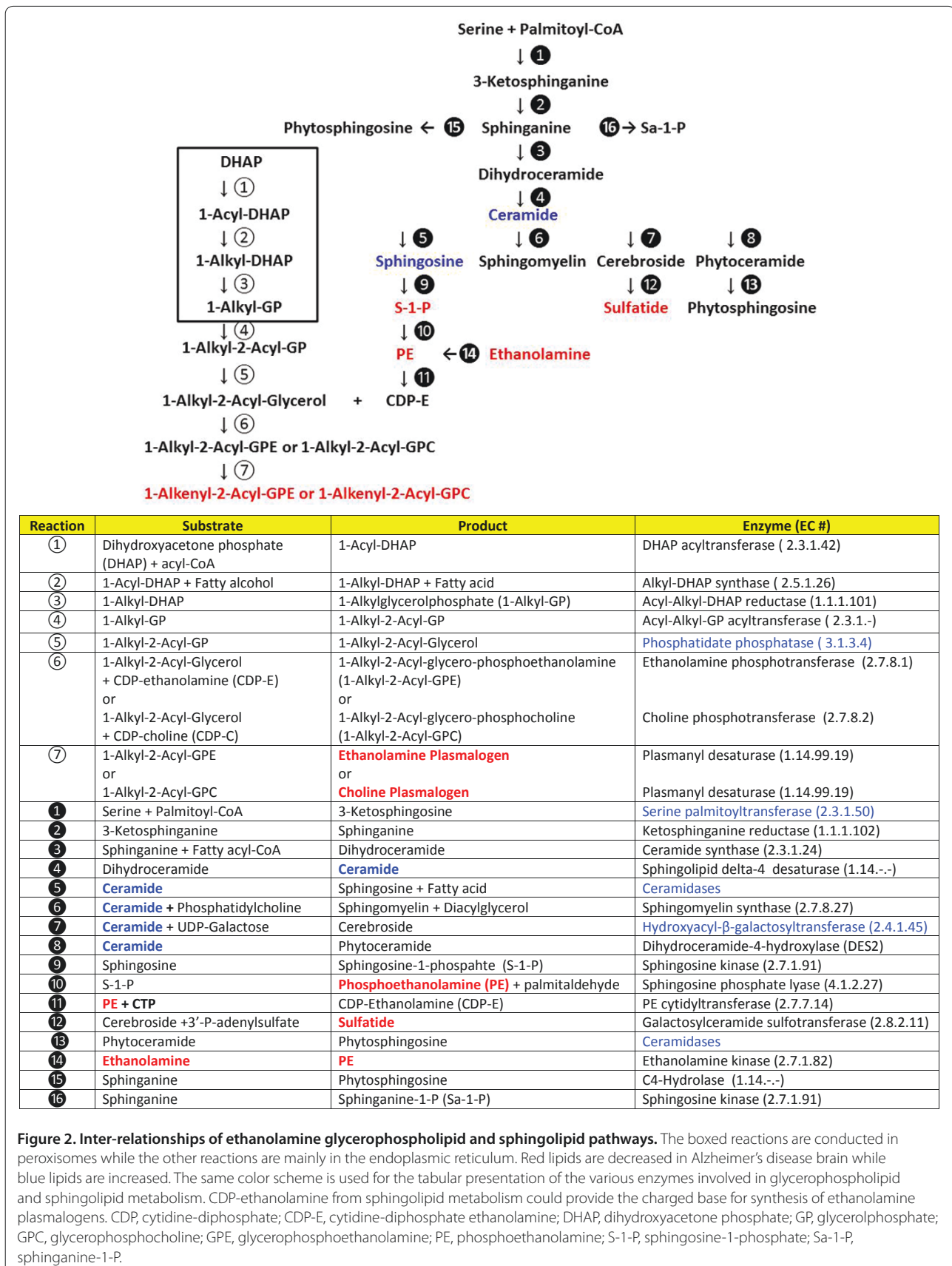|	Lipid	R	R'	R''	Composition
CH₂-R | CH-O-R' | CH₂-O-R''	Glycerophosphethanolamine	OH	H	PEtn	$C_5H_{15}NO_6P$ 216.1
	Glycerophosphocholine	OH	H	PCh	$C_8H_{20}NO_6P$ 257.1
	Diacylglycerol	O-CO-18:0	22:6	H	$C_{43}H_{72}O_5$ 668.5
	1-Alkyl-2-acyl-glycerol	O-18:0	22:6	H	$C_{43}H_{70}O_5$ 666.5

Figure 1. Chemical structures of glycerophospholipids. Phosphatidyl glycerophospholipids are diacyl lipids in which fatty acids are the substituents at sn-1 and sn-2 and charged bases (PEtn, PCh, PSer or PIn) at sn-3. Ether lipids possess a fatty alcohol at sn-1 and a fatty acid at sn-2. In plasmalogens, the fatty alcohol at sn-1 possesses a desaturation at the first two carbons in the fatty alcohol substitution. Loss of a fatty acid generates the lysophosphatidyl glycerophospholipids, via deacylation at sn-2 of diacyl glycerophospholipids.

Parkinson's substantia nigra and demonstrated anatomic specificity, being marked in the mid-temporal cortex but not the cerebellum [8,9]. These deficiencies in a major structural phospholipid pool were rapidly validated by other research groups and quantification of individual PlsEtns by tandem mass spectrometry demonstrated that white matter PlsEtns (that is, oleic or linoleic acid at sn-2; Figure 1) were decreased by up to 40% early in the disease process [9,10]. Gray matter PlsEtns (that is, docosa-hexaenoic (DHA) or arachidonic acid at sn-2; Figure 1) decreased in a disease severity-dependent manner [9]. Of particular note, while there were dramatic decreases in cerebellar white matter PlsEtns, there were no changes in cerebellar gray matter PlsEtns even in late stage AD [9]. PlsEtn changes were also shown to be specific in that phosphatidylethanolamines, serine glycerophospholipids and inositol glycerophospholipids were unaltered [8-10] (nomenclature and structures are presented in Figure 1).

Subsequent research demonstrated disease severity-dependent decreases in circulating DHA and PlsEtns, particularly PlsEtns containing DHA at sn-2 [11,12]. While plasma DHA is derived both from the diet and from peroxisome-dependent synthesis by the liver and gastrointestinal epithelium, peroxisomes are obligatory

Reaction	Substrate	Product	Enzyme (EC #)
①	Dihydroxyacetone phosphate (DHAP) + acyl-CoA	1-Acyl-DHAP	DHAP acyltransferase (2.3.1.42)
②	1-Acyl-DHAP + Fatty alcohol	1-Alkyl-DHAP + Fatty acid	Alkyl-DHAP synthase (2.5.1.26)
③	1-Alkyl-DHAP	1-Alkylglycerolphosphate (1-Alkyl-GP)	Acyl-Alkyl-DHAP reductase (1.1.1.101)
④	1-Alkyl-GP	1-Alkyl-2-Acyl-GP	Acyl-Alkyl-GP acyltransferase (2.3.1.-)
⑤	1-Alkyl-2-Acyl-GP	1-Alkyl-2-Acyl-Glycerol	Phosphatidate phosphatase (3.1.3.4)
⑥	1-Alkyl-2-Acyl-Glycerol + CDP-ethanolamine (CDP-E) or 1-Alkyl-2-Acyl-Glycerol + CDP-choline (CDP-C)	1-Alkyl-2-Acyl-glycero-phosphoethanolamine (1-Alkyl-2-Acyl-GPE) or 1-Alkyl-2-Acyl-glycero-phosphocholine (1-Alkyl-2-Acyl-GPC)	Ethanolamine phosphotransferase (2.7.8.1) Choline phosphotransferase (2.7.8.2)
⑦	1-Alkyl-2-Acyl-GPE or 1-Alkyl-2-Acyl-GPC	**Ethanolamine Plasmalogen** or **Choline Plasmalogen**	Plasmanyl desaturase (1.14.99.19) Plasmanyl desaturase (1.14.99.19)
❶	Serine + Palmitoyl-CoA	3-Ketosphingosine	Serine palmitoyltransferase (2.3.1.50)
❷	3-Ketosphinganine	Sphinganine	Ketosphinganine reductase (1.1.1.102)
❸	Sphinganine + Fatty acyl-CoA	Dihydroceramide	Ceramide synthase (2.3.1.24)
❹	Dihydroceramide	**Ceramide**	Sphingolipid delta-4 desaturase (1.14.-.-)
❺	**Ceramide**	Sphingosine + Fatty acid	Ceramidases
❻	**Ceramide** + Phosphatidylcholine	Sphingomyelin + Diacylglycerol	Sphingomyelin synthase (2.7.8.27)
❼	**Ceramide** + UDP-Galactose	Cerebroside	Hydroxyacyl-β-galactosyltransferase (2.4.1.45)
❽	**Ceramide**	Phytoceramide	Dihydroceramide-4-hydroxylase (DES2)
❾	Sphingosine	Sphingosine-1-phospahte (S-1-P)	Sphingosine kinase (2.7.1.91)
❿	S-1-P	**Phosphoethanolamine (PE)** + palmitaldehyde	Sphingosine phosphate lyase (4.1.2.27)
⓫	**PE** + CTP	CDP-Ethanolamine (CDP-E)	PE cytidyltransferase (2.7.7.14)
⓬	Cerebroside +3'-P-adenylsulfate	**Sulfatide**	Galactosylceramide sulfotransferase (2.8.2.11)
⓭	Phytoceramide	Phytosphingosine	Ceramidases
⓮	**Ethanolamine**	**PE**	Ethanolamine kinase (2.7.1.82)
⓯	Sphinganine	Phytosphingosine	C4-Hydrolase (1.14.-.-)
⓰	Sphinganine	Sphinganine-1-P (Sa-1-P)	Sphingosine kinase (2.7.1.91)

Figure 2. Inter-relationships of ethanolamine glycerophospholipid and sphingolipid pathways. The boxed reactions are conducted in peroxisomes while the other reactions are mainly in the endoplasmic reticulum. Red lipids are decreased in Alzheimer's disease brain while blue lipids are increased. The same color scheme is used for the tabular presentation of the various enzymes involved in glycerophospholipid and sphingolipid metabolism. CDP-ethanolamine from sphingolipid metabolism could provide the charged base for synthesis of ethanolamine plasmalogens. CDP, cytidine-diphosphate; CDP-E, cytidine-diphosphate ethanolamine; DHAP, dihydroxyacetone phosphate; GP, glycerolphosphate; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; PE, phosphoethanolamine; S-1-P, sphingosine-1-phosphate; Sa-1-P, sphinganine-1-P.

for PlsEtn synthesis [13]. Based on these data, a peroxisomal deficit in AD was proposed [11] and was soon demonstrated in AD liver [14] and AD brain [15,16]; however, these data do not also preclude a contribution of lipid peroxidation in plasmalogen decrements or potential drug-induced liver toxicity. Brain and liver DHA is decreased in AD tissues and pristanic acid, a metabolite of dietary phytanic acid that is metabolized exclusively by peroxisomes [17], is elevated in AD liver [14]. Decreases in AD brain PlsEtn levels are accompanied by accumulation of very long chain fatty acids (VLCFAs): behenic acid (C22:0), lignoceric acid (C24:0) and hexacosanoic acid (C26:0) [15]. These VLCFAs are all metabolized by peroxisomes [13,15], again supporting peroxisomal dysfunction in AD. Furthermore, the decrements in brain [9], liver [14] and plasma [11,12] DHA-containing plasmalogens and the accumulation of VLCFAs in the cortex [15] correlate with cognitive deficit in AD patients. In contrast, decreases in white matter PlsEtns occur early in the disease process and do not correlate with cognitive status [9].

Supply of PlsEtn building blocks to the central nervous system (CNS) is complicated in that a number of lipid transport mechanisms are involved. DHA (omega-3) and arachidonic acid (omega-6), which are long chain polyunsaturated fatty acids essential for PlsEtn synthesis, comprise 8% and 6% of brain dry weight, respectively [1]. Fatty acid binding proteins [18] are major determinants of fatty acid transport into and within the CNS. The heart type fatty acid binding protein (FABP3) has been evaluated in AD and found to be decreased in brain and plasma, but increased in CSF [18]. Epidermal fatty acid binding protein (FABP5) and brain fatty acid binding protein (FABP7), both present in the brain, remain to be evaluated.

Circulating plasmalogens and plasmalogen precursors also gain access to the CNS. Circulating plasmalogens are synthesized mainly by the liver and gastrointestinal epithelium [14,19] and are exported into the circulation via chaperone transport proteins of which low density lipoprotein (LDL) is a major carrier, containing micromolar concentrations of PlsEtns [20]. These chaperone proteins are critical since free plasmalogens are metabolically unstable. In the case of the blood-brain barrier and blood-retinal barrier, transport of the plasmalogens is via an LDL receptor-mediated transcytosis pathway that bypasses the lysosomal compartment [21]. This transport pathway preferentially shuttles LDL enriched in DHA-containing phospholipids [22]. LDL receptor function in AD and its potential impact on supply of plasmalogens to the CNS remain to be more clearly defined.

In summary, there are a number of significant decrements in brain polyunsaturated fatty acids and PlsEtns in AD. These include early and dramatic decreases in white

matter PlsEtns in the brain and a disease severity-dependent decrease in gray matter PlsEtns. These changes in lipid dynamics appear to be the result of peroxisomal dysfunction in both the liver [14] and brain [15,16], a conclusion further supported by the accumulation of VLCFAs in AD brain [15]. Since circulating plasmalogens are decreased in a number of other clinical conditions, the effects of these potential confounds need to be addressed in future AD studies. These include plasmalogen decrements in ischemic cerebrovascular disease [23], Parkinson's disease [24], hypertension [25], uremia [26], and hyperlipidemia [27].

Choline glycerophospholipids

Choline is an essential precursor for the synthesis of glycerophosphocholines (GPCs). However, choline levels are affected dramatically by agonal status and post-mortem delays in human tissue handling. The net result is that there is no consistent finding from publications of choline levels in AD brain. However, as observed with PlsEtns, it appears there are decrements in brain choline plasmalogens (PlsChs; Figure 1) [28] but not in phosphatidylcholines [10]. These analyses were not performed with liquid chromatography-tandem mass spectrometry such that individual PlsChs were not characterized but fatty acid analyses did demonstrate deficits in the total DHA-containing PlsCh pool [28]. The PlsCh metabolite GPC has been shown to be increased in AD cortex [6] and CSF [29]. The accumulation of GPC is potentially indicative of increased degradation of choline glycerophospholipids and/or decreased GPC metabolism. Decreases in AD cortical phospholipase D [30], an enzyme that removes choline from GPC and lysophosphatidylcholines (LPCs), may also contribute to GPC accumulation. However, GPC phosphodiesterase (EC 3.1.4.2, EC 3.1.4.46), another enzyme that metabolizes GPC, is increased in AD cortex but not cerebellum [31]. In a non-targeted metabolomics study of AD plasma, decrements in LPC 16:0 (palmitic acid) and LPC 18:2 (linoleic acid) have been reported [32]. The anatomical source(s) of these circulating metabolites remains to be defined.

In summary, it appears that peroxisomal deficits in AD [14-16] result in decreases in brain PlsChs and alterations in the metabolism of GPCs. The impact of alterations in the metabolism of GPCs on cholinergic neurotransmission remain to be investigated. These early autopsy studies need to be repeated and the individual PlsChs characterized, while studies of plasma LPCs require larger population-based studies.

Glycerophospholipid remodeling

While brain glycerophospholipids possess almost exclusively palmitic (16:0), stearic (18:0) or oleic acid (18:1) at

sn-1, the fatty acid substitution at sn-2 is much more varied. In white matter, the sn-2 position is dominated by oleic acid while in gray matter DHA (22:6) and arachidonic acid (20:4) predominate. A further critically important feature of the sn-2 fatty acid substitutions is that they are dynamic, undergoing continuous remodeling. Lipid remodeling is a process involved in the generation of a large family of PlsEtns and PlsChs in the brain. The lipid remodeling pathway involves removal of sn-2 fatty acids by the 2-acyl hydrolases, phospholipase A2 (EC 3.1.1.4) and acylglycerol lipase (EC 3.1.1.23) and reacylation, with alternative fatty acids, by acyl-CoA:lysophospholipid 2-acyltransferases [33].

Of the 22 different phospholipase A2 (PLA2) enzymes, several have been evaluated in AD. Plasmalogen-selective PLA2 [34] and cytosolic PLA2 [35] have been reported to be increased in AD cortex. Ca^{++}-dependent PLA2 is increased in AD CSF [36], decreased in AD cortex [31], and unaffected in AD cerebellum [31]. Ca^{++}-independent PLA2 is decreased in both AD CSF [37] and AD cortex [31], and unaffected in AD cerebellum [31]. Clearly, further characterization of changes in the multiple isoforms of PLA2 in AD is required. In parallel with increases in AD cortical plasmalogen-selective PLA2 [34], lysophospholipid acyltransferase is increased [31], further supporting the tight coupling of deacylation-reacylation in lipid remodeling. This coordinated deacylation-reacylation mechanism appears to be located mainly in the endoplasmic reticulum [33], also the location of the final steps of plasmalogen and sphingo-lipid synthesis (Figure 2).

A major aspect of lipid remodeling that remains to be evaluated in AD are the signaling cascades potentially evoked by lipids released by deacylation at sn-2 of glycerophospholipids. In the case of released arachidonic acid, a vast array of eicosanoids can be generated as mediators of neuroinflammation, a common feature of AD brain [38]. In contrast, released DHA can be utilized in the generation of anti-inflammatory and neuroprotective docosanoids [39], lipid metabolites that remain to be investigated in AD brain.

In summary, studies of lipid remodeling in AD brain indicate that these processes are augmented and further contribute to changes in glycerophospholipid dynamics in AD and in the processing of amyloid [40].

Sphingolipids

Sphingolipids are major structural lipids of CNS membranes and in the case of sulfatides (Figure 3) are highly expressed in myelin. Sphingolipids constitute 5 to 7% of the myelin lipid pool and are synthesized by oligo-dendrocytes. Early reports of large decrements in white matter sulfatides [4] were validated and individual sulfatides characterized by tandem mass spectrometry [41]. The major sulfatide pools in the human CNS include D18:1 (sphingosine)/24:1 (nervonic acid), D18:1/24:1h (α-hydroxynervonic acid; cerebronic acid), and D18:1/26:1 (hexacosenoic acid), (Figure 3). While sulfatide levels are decreased in AD cortex, the compositional distribution of sulfatide subtypes is unaltered in AD brain [42]. Sulfatide depletion is up to 93% in the gray matter and occurs early in the disease process while sulfatide depletion is disease severity-dependent in white matter, with up to 58% depletion [41]. Sulfatide losses in AD cortex are disease specific in that they do not occur in subjects with Parkinson's disease, dementia with Lewy bodies, frontotemporal dementia, or multiple sclerosis [43]. Sulfatide synthesis (Figure 2) does not appear to be altered in that the pivotal synthetic enzyme galacto-cerebroside sulfotransferase (Figure 2, reaction 12) is normal in AD postmortem cortex, as are levels of cerebrosides, the direct precursors of sulfatides [10]. In contrast to AD cortex, recent studies of AD hippocampus have reported decrements in the total cerebroside pool, while cerebrosides with 2-hydroxylated fatty acids (for example, cerebronic acid) are slightly increased [44]. These changes are specific to the hippocampus and are not seen in the cerebellum [44]. Whether these differences between cortex and hippocampus represent a true regional difference or if the relative mix of white and gray matter sampled is responsible for these differences remains to be investigated.

Decreases in AD cortical sulfatides are paralleled by large increases in cortical [41,45,46] and CSF [47] ceramides, which are precursors/degradation products of sulfatides (Figure 3). White matter ceramides are increased three-fold in the AD temporal cortex and cerebellum early in the disease process [41]. In late stage AD, these increases in white matter ceramides remain elevated at two-fold that of age-matched controls while gray matter ceramides are unaltered at all stages of AD [41]. Immunohistochemistry studies suggest that the increases in cortical ceramides are mainly in astrocytes [47]. The major ceramide that increases is N24:1 (nervonic acid) ceramide (Figure 3), the precursor/degradation product of the predominant brain sulfatide D18:1 (sphingo-sine)/24:1 (nervonic acid) [41].

Increases in ceramide levels are paralleled by increases in both acid ceramidase (EC 3.5.1.23) [46,48] and galactosylceramidase (EC 3.2.1.46) [49]. Augmentation of brain ceramidase levels is most likely the basis of increased brain levels of sphingosine (Figure 2, reaction 5) in AD [49]. In contrast, sphingosine-1-phosphate is decreased in AD brain [46]. The potential signaling effects of alterations in brain ceramides, sphingosine and sphingosine-1-phosphate remain to be determined. One of the established actions of ceramides is the activation of brain plasmalogen-selective PLA2 [50], thereby potentially
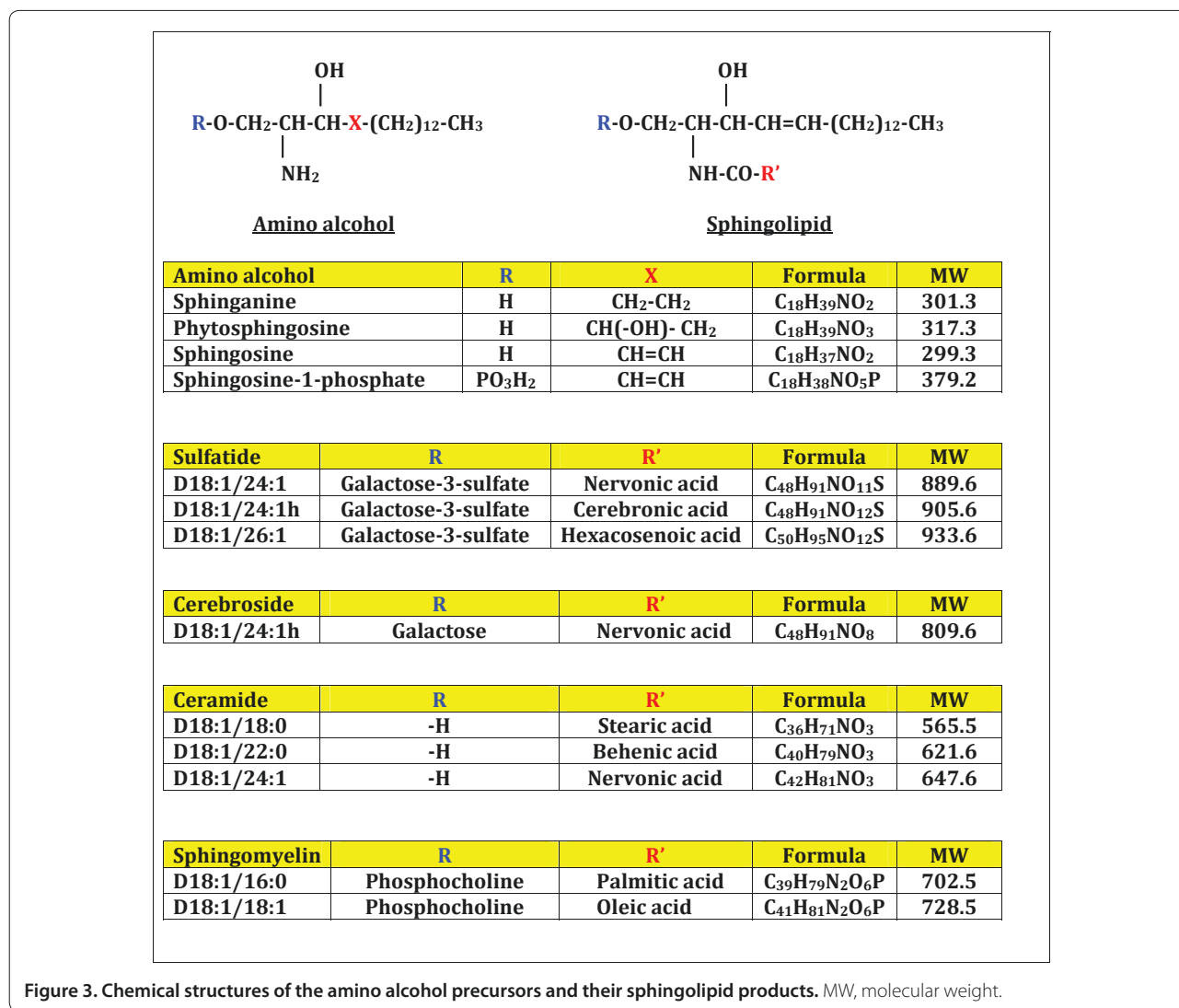
$$OH$$
$$|$$
$$R\text{-O-CH}_2\text{-CH-CH-X-(CH}_2)_{12}\text{-CH}_3$$
$$|$$
$$NH_2$$

Amino alcohol

$$OH$$
$$|$$
$$R\text{-O-CH}_2\text{-CH-CH-CH=CH-(CH}_2)_{12}\text{-CH}_3$$
$$|$$
$$NH\text{-CO-R'}$$

Sphingolipid

Amino alcohol	R	X	Formula	MW
Sphinganine	H	CH_2-CH_2	$C_{18}H_{39}NO_2$	301.3
Phytosphingosine	H	CH(-OH)- CH_2	$C_{18}H_{39}NO_3$	317.3
Sphingosine	H	CH=CH	$C_{18}H_{37}NO_2$	299.3
Sphingosine-1-phosphate	PO_3H_2	CH=CH	$C_{18}H_{38}NO_5P$	379.2

Sulfatide	R	R'	Formula	MW
D18:1/24:1	Galactose-3-sulfate	Nervonic acid	$C_{48}H_{91}NO_{11}S$	889.6
D18:1/24:1h	Galactose-3-sulfate	Cerebronic acid	$C_{48}H_{91}NO_{12}S$	905.6
D18:1/26:1	Galactose-3-sulfate	Hexacosenoic acid	$C_{50}H_{95}NO_{12}S$	933.6

Cerebroside	R	R'	Formula	MW
D18:1/24:1h	Galactose	Nervonic acid	$C_{48}H_{91}NO_8$	809.6

Ceramide	R	R'	Formula	MW
D18:1/18:0	-H	Stearic acid	$C_{36}H_{71}NO_3$	565.5
D18:1/22:0	-H	Behenic acid	$C_{40}H_{79}NO_3$	621.6
D18:1/24:1	-H	Nervonic acid	$C_{42}H_{81}NO_3$	647.6

Sphingomyelin	R	R'	Formula	MW
D18:1/16:0	Phosphocholine	Palmitic acid	$C_{39}H_{79}N_2O_6P$	702.5
D18:1/18:1	Phosphocholine	Oleic acid	$C_{41}H_{81}N_2O_6P$	728.5

Figure 3. Chemical structures of the amino alcohol precursors and their sphingolipid products. MW, molecular weight.

contributing to the decrements in brain plasmalogens in AD (see the 'Choline glycerophospholipids' section). While these studies of AD cortex are consistent in their observations of elevated ceramide levels, analysis of AD hippocampus has revealed decrements in ceramide levels [44]. Clearly, more detailed regional AD brain analyses are needed as well as more detailed comparisons of mild cognitive impairment (MCI) and AD disease stages.

Plasma lipidomics studies have also demonstrated increased ceramide levels in AD patients. These changes include increases in N16:0 (palmitic acid) and N21:0 (heneicosanoic acid) ceramides [51]. In contrast, another study has reported no differences in plasma ceramide levels in AD [52] but decreased plasma levels in MCI. The major ceramide species that are decreased in MCI patients contain the long chain saturated fatty acids C22:0 (behenic acid), C24:0 (lignoceric acid), and C26:0 (cerotic acid) [52]. The ceramide precursor sphinganine and its metabolite phytosphinganine (Figure 2; Figure 3) have also been reported to be decreased in AD plasma [32]. All of these studies are based on small patient numbers, and larger population studies are clearly needed to resolve the reported differences.

In contrast to brain, which demonstrates no alterations in sphingomyelin levels [10], decrements in plasma sphingomyelins have been monitored, particularly sphingomyelins with very long chain monounsaturated fatty acid substitutions [51]. These include sphingomyelin D18:1 (sphingosine)/24:1 (nervonic acid) and D18:1/22:1 (erucic acid). In addition, longitudinal studies over a 2.3 year period have demonstrated that higher plasma levels of sphingomyelins are predictors of slower disease progression in AD patients [53].

In summary, brain sphingolipid metabolism is dramatically affected early in AD white and gray matter. Decrements in myelin sulfatides support imaging studies

demonstrating hypomyelination in MCI and AD patients. These sulfatide losses appear to involve both increased degradation and increased export from oligodendrocytes [42,48]. Clearly, dramatic alterations in brain sphingolipid metabolism occur early in the pathogenesis of AD. The reliability/utility of reported changes in plasma sphingolipids remain to be validated in larger and more diverse patient populations.

Pathophysiology of glycerophospholipid and sphingolipid changes in AD

Myelin and neuronal membrane structural defects
Myelin is extremely rich in both plasmalogens and sulfatides as structural lipids. PlsEtns encompass 22.4% of plasmalogens in myelin, in contrast to choline plasmalogens, which comprise only 0.9% of that mass [13]. In addition to myelin, PlsEtns are major structural phospholipids in neuronal membranes and mitochondria, comprising about 30% of total phospholipids and 85% of ethanolamine phospholipids [9]. Sulfatides account for 5% of myelin lipids and are critical to paranodal junction formation. The combined loss of myelin sulfatides and PlsEtns further reflects a complex and progressive hypomyelination process in AD. This hypomyelination is further demonstrated by decrements in protein biomarkers of myelin integrity, namely 2',3'-cyclic nucleotide-3'-phosphodiesterase [54] and myelin basic protein [55]. Imaging studies in MCI and AD patients have consistently demonstrated microstructural changes in myelin. In particular, decreased myelin integrity appears to be most marked in late-myelinating fiber tracts, such as the inferior- and superior-longitudinal fasciculi [56]. This hypomyelination combined with decreased supply of neuronal plasmalogens also can explain the dramatic shrinkage and dysfunction of AD cortical neurons and the nucleus basalis-cortex cholinergic projection [57-59], which is critical in cognitive function.

Structural lipid deficits and signal transduction
Sphingolipids and sphingolipid metabolites are important mediators of signal transduction in the CNS, as they act on membrane-associated receptors and are precursors for a number of bioactive lipids. Ceramides are thought to participate in neuroinflammation and in activation of cell death pathways [41,45]. Ceramides are known to induce a number of inflammatory cytokines, including IL-6, which is dramatically elevated in AD brain [38]. Deregulation of sphingolipid metabolism may also have dramatic effects on nuclear function. Sphingomyelin is the major sphingolipid in nuclei and is involved in chromatin architecture, RNA stability and DNA synthesis [60]. Sphingosine and sphingosine-1-phosphate, metabolites of sphingomyelin, also regulate gene transcription and histone acetylation, respectively [60]. The impact of

alterations in the dynamics of sphingolipids on these functions in AD remains to be investigated.

Plasmalogens are major structural phospholipids in membranes, such that changes in their concentrations and/or composition will dramatically affect membrane fluidity, thereby negatively affecting the functions of membrane transporters, ion channels and membrane-bound enzymes [13,59]. Alterations in lipid remodeling [33] also will affect signal transduction via alterations in the concentrations of free arachidonic acid and DHA available for the synthesis of eicosanoids and docosanoids, respectively [39].

Structural lipid deficits and cholinergic function
The cholinergic hypothesis of cognitive dysfunction in AD remains a core concept in AD research [59]. Understanding the pathophysiology of this neurotransmitter deficit is at the core of many research group's efforts in defining potential new therapeutic approaches for AD. Based upon our current knowledge of lipidomics changes in the AD brain, the following points can be summarized about the possible negative contributions of lipid deficits on cholinergic function.

First, decrements in sulfatides and plasmalogens, resulting in hypomyelination and membrane dysfunction, may be responsible for the marked shrinkage of nucleus basalis-cortical cholinergic neurons [57-59].

Second, hypomyelination is known to reduce axonal transport in AD cortex [61]. Decreased anterograde transport of choline acetyltransferase and the acetylcholine vesicular transporter would reduce the synthetic and storage capacities for acetylcholine in the cortical nerve terminal fields [59]. Decreased retrograde transport of essential trophic factors from the cortex to the cholinergic cell bodies in the basal forebrain would lead to decreased trophic support and decreased cell body synthesis of critical proteins required for cholinergic neuronal function [59].

Third, decreased availability of plasmalogens to cholinergic nerve terminal would impact mitochondrial function and neurotransmitter vesicular fusion with the presynaptic membrane, both resulting in decreased synaptic acetylcholine release [59]. Accumulation of VLCFAs [15] also will negatively affect mitochondrial function.

Fourth, decreased plasmalogens in AD membrane lipid rafts [62] would alter membrane fluidity uncoupling cholinergic muscarinic receptors in AD cortex [63], as has been shown to occur in plasmalogen-deficient cell lines [64]. Decreased acetylcholine release coupled with impaired postsynaptic signal transduction may well be responsible for the marked cholinergic deficit in AD patients [59].

Fifth, deficits in sulfatides and plasmalogens also will negatively affect gap junction integrity, thereby impairing

glial metabolic support of cholinergic nerve terminals in the cortex [59].

Sixth, decreases in membrane plasmalogen levels result in decreased export of cholesterol, further contributing to membrane rigidity [59,65]. This increase in membrane rigidity is hypothesized to contribute to abnormal processing of β-amyloid in AD [59,65].

Seventh, neuroinflammation is a hallmark feature of AD brain [38,59]. In this regard, neuroinflammation has been demonstrated to be induced by metabolites of sphingolipids and by decrements in membrane plasmalogens, both features of AD brain [59].

Eighth, astroglial-neuronal and neuronal-neuronal exchange of exosomes may be altered since these secretory vesicles have a lipid composition similar to lipid rafts, being rich in cholesterol, sphingomyelin and plasmalogens. Decreases in these communication pathways [66] may also negatively affect cholinergic neurotransmission.

Conclusion

It is now clear that peroxisomal dysfunction in AD leads to deficits in both ethanolamine and choline plasmalogens and accumulation of VLCFAs. These alterations have the potential to be major determinants in neuronal dysfunction in AD. Alterations in the dynamics of sphingolipids are just becoming more understood and presumably underlie the myelin abnormalities that have been reported in AD. Integrating lipidomics findings to date, it appears that in white matter there are about 58% depletions in sulfatides, three-fold increases in ceramides and 40% decreases in PlsEtns early in the disease process (Clinical Dementia Rating of 0.5) and that these changes are sustained in advanced disease (Clinical Dementia Rating of 3.0) [9,10]. In contrast, sulfatides are depleted by 93%, ceramides are unaltered and PlsEtns demonstrate a disease severity-dependent decrease in AD gray matter [9,41]. These data indicate that alterations in glycerophospholipids and sphingolipids occur early in the AD process and probably involve a number of factors related to lipid synthesis, transport and degradation.

The challenges ahead are to evaluate plasma lipidomics in larger and more diverse patient populations to determine their utility in disease diagnosis and in monitoring disease progress. More detailed regional lipidomics analysis of AD brain samples is required as well as in-depth comparisons at different disease stages. A further comparison that has high probability of yielding very valuable data will be to investigate the lipidomics of brain samples from individuals with no cognitive impairment demonstrating significant AD neuropathological burden [67]. Understanding the differences in lipidomics between no-cognitive-impairment and AD brains will help pinpoint critical metabolic changes that result in cholinergic dysfunction. These approaches will define the utility

of glycerophospholipids and sphingolipids as potential biomarkers of disease progression and help define new points of pharmacological intervention for the treatment of AD.

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