CMLS, Cell. Mol. Life Sci. 61 (2004) 1896–1906
1420-682X/04/151896-11
DOI 10.1007/s00018-004-4009-z

CMLS Cellular and Molecular Life Sciences

Review

The role of apolipoprotein E in lipid metabolism in the central nervous system

X. Han

Division of Bioorganic Chemistry and Molecular Pharmacology, Department of Medicine, Washington University School of Medicine, Box 8020, 660 South Euclid Avenue, St. Louis, Missouri 63110 (USA), Fax: +1 314 362 1402, e-mail: xianlin@wustl.edu

Received 9 January 2004; received after revision 28 February 2004; accepted 10 March 2004

Abstract. The critical roles of apolipoprotein E (apoE) in regulating plasma lipid and lipoprotein levels have been extensively studied for over 2 decades. However, an understanding of the roles of apoE in the central nervous system (CNS) is less certain. This review will summarize the available experimental results on the role of apoE in CNS lipid homeostasis with respect to its modulation of sulfatide trafficking, alteration of CNS cholesterol homeostasis and apoE-induced changes in phospholipid molecular species in specialized subcellular membrane fractions. The results indicate that apoE mediates sulfatide trafficking and metabolism in the CNS. Moreover, although apoE does not affect the cholesterol mass content or the phospholipid mass levels and composition in the CNS as a whole, apoE modulates cholesterol and phospholipid homeostasis in selective subcellular membrane compartments. Through elucidating the roles of apoE in CNS lipid metabolism, new insights into overall functions of apoE in neurobiology can be accrued ultimately, leading to an increased understanding of CNS lipid metabolism and the identification of novel therapeutic targets for CNS diseases.

Key words. Alzheimer's disease; apolipoprotein E; cholesterol; lipidomics; electrospray ionization mass spectrometry; phospholipids; sulfatide.

Introduction

The intercellular transport of lipids through the circulatory system requires the packaging of hydrophobic molecules into water soluble carriers (lipoproteins) and the regulated targeting of these molecules to appropriate tissues by receptor-mediated endocytic pathways as well as scavenger receptor-mediated pathways. Plasma lipoproteins have been classified into five major groups based on their size and density. These include chylomicrons, very low density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) [1]. Chylomicrons and VLDL possess high lipid:protein ratios, and are the major carriers of triglycerides. IDL and LDL are smaller particles that contain large amounts of cholesterol and cholesterol esters; in humans, LDL are the principal cholesterol-transporting lipoproteins in plasma [2]. HDL are the smallest of these particles and contain the highest protein/lipid ratio. HDL can be further subfractioned into HDL_2 and HDL_3. Specific types of HDL particles are involved in the process of 'reverse cholesterol transport', a pathway whereby these particles acquire cholesterol from peripheral tissues and transport it to the liver for excretion in the bile (see [3–5] for recent reviews). In addition to the presence of lipoproteins in the plasma, lipoproteins are also present in cerebrospinal fluid (CSF), the interstitial space of the brain and in other body fluids. CSF lipoproteins are predominantly the size and density of plasma HDL [6, 7], although they may have distinct functions from plasma HDL, such as participation in reverse cholesterol transport.

Each type of lipoprotein has distinct protein (called apolipoprotein) and lipid constituents, and varying physiological activities as a result of this compositional heterogeneity. Apolipoproteins present in lipoprotein particles function to (i) mediate binding of the lipoproteins to cell-surface receptors; (ii) act as cofactors for enzymes of lipid metabolism and (iii) maintain the structural integrity of lipoprotein particles as they are transported [8]. The soluble apolipoprotein gene family includes apolipoproteins E and J (apoE and apoJ), which encode proteins with amphipathic α-helical structures in the C-terminus that allow these proteins to exist at the lipoprotein particle surface [9]. CSF lipoproteins appear to be heterogeneous in apolipoprotein content, with apoE localized to the largest particles, apoAI and apoAII localized to smaller particles that may originate from the plasma, and apoJ distributed among the entire particle size range [6]. The metabolism of CNS lipoproteins and its potential relationship with the neurodegenerative diseases has recently been extensively reviewed [6, 10].

Human apolipoprotein E (apoE, protein; *APOE*, gene) is a 299-amino acid (34-kDa) protein. The human *APOE* gene is located on the long arm of chromosome 19q13.2, and numerous mutations and polymorphisms within the exons, introns and the promotor region have been described [11–13]. There are three major isoforms of human apoE that differ in amino acid composition at positions 112 and 158 [14]. The most common isoform, apoE3, has cysteine at position 112 and arginine at 158 and is considered to be the 'normal' form of apoE. ApoE2 and apoE4 occur less frequently, differing from apoE3 by only a single amino acid. ApoE2 has cysteine at both 112 and 158 positions, whereas apoE4 contains arginine at both positions. Linkage analysis and/or association studies on the genes playing a role in longevity revealed that *APOE* has given the most reproducible and strongest association with longevity (see [15] for recent review).

ApoE messenger RNA (mRNA) is predominantly expressed in the liver [16] with the brain having the next highest levels of expression [17]. For example, a level of 220–280 pg/µg RNA is present in mouse liver, and 35–40 pg/µg RNA is contained in mouse brain [18]. Plasma apoE is primarily derived from liver parenchymal cells (at least three-fourths of circulating apoE) and, to a much lesser extent, from macrophages throughout the body [16, 19]. ApoE in the CNS is known to be derived from the brain and not from the liver [20]. ApoE mRNA in the CNS is predominantly synthesized by astrocytes [21, 22]. Why apoE is predominantly expressed in astrocytes remains an unanswered question in neuronal cell biology. However, evidence from multiple recent studies supports a hypothesis that apoE expression in astrocytes is the result of lipid metabolic cooperation between neurons and astrocytes that allows neurons to specialize in electrical signaling [23]. Additional studies will be required to verify this intriguing hypothesis.

ApoE is a constituent of several classes of plasma lipoproteins, including chylomicrons, VLDL and HDL. ApoE plays a prominent role in the transport and metabolism of plasma cholesterol, triacylglycerols and phospholipids among various cells of body [14]. This role is achieved through its ability to interact with the LDL receptor and the LDL receptor-related protein [14, 24]. Understanding of the role of apoE in lipid metabolism was greatly advanced by the discovery that apoE2 is defective in lipoprotein receptor binding and is genetically associated with type III hyperlipoproteinemia (see [25, 26] for reviews).

ApoE is one of the primary apolipoproteins in the CNS [7, 27, 28], mediating the transport of cholesterol, phospholipids and sulfatides [29–31] and playing a key role in neurobiology (see [13, 32, 33] for recent reviews). In vitro studies utilizing neuronal models of the central and peripheral nervous systems revealed that apoE can promote neurite outgrowth [17, 34–37] and that this effect is lipid dependent [35]. Furthermore, it has been speculated that apoE plays a role in the development, remodeling and regeneration of the nervous system in animal models [38–41], but apoE may be not essential for nervous regeneration in the peripheral nervous system [42]. ApoE4 has been shown to be a major known risk factor of AD (see [43, 44] for reviews). However, the role of apoE in the pathogenesis of AD is not entirely clear. Recent studies suggest that apoE influences amyloid-β (Aβ) deposition and toxicity in the brain [45–49]. Current evidence has also identified the ε4 allele as a major risk factor for the poor clinical outcome of certain forms of brain injury, including that due to head trauma [50–53], spontaneous intracerebral hemorrhage [54, 55] and possibly stroke [56]. ApoE4 also appears to influence the age of onset of Parkinson's disease [57]. To date, the mechanisms of how apoE is involved in these biological processes are unclear (see [33] for recent review).

Studies with peripheral nerves in vitro suggested a model in which apoE may function in lipid transport following nerve injury and assist in the growth and remyelination of damaged neural processes (ensheathment by Schwann cell processes and their lipid-rich membrane) [58]. For example, following peripheral nerve injury, lipids and cholesterol are removed from damaged neural and glial processes and associate with apoE-containing lipoproteins. Over time, it appears that these apoE-containing lipoproteins are then able to deliver cholesterol and lipid to regenerating neural and glial processes for synthesis of new membrane [59]. Handelmann and colleagues [60] provided evidence that apoE and cholesterol could directly play a role in stimulating neural process (axon/dendrite) growth and remodeling of the peripheral nervous system. In that study, cholesterol- and apoE-enriched

β-VLDL increased rabbit neurite outgrowth (neurite length) and branching of dorsal root ganglion neurons in culture, while nonesterified cholesterol had similar but less pronounced effects. This suggested that cholesterol delivery might be responsible for the neurite-promoting effects. Furthermore, addition of β-VLDL that had been enriched with rabbit apoE resulted in greater neurite extension with less neurite branching than with β-VLDL alone, indicating that β-VLDL may facilitate neurite extension by promoting cholesterol uptake, a process known to be facilitated by apoE. Because it has been demonstrated that β-VLDL is a ligand for LDLR and apoE-enriched β-VLDL is a ligand for LDL receptor and the LDL receptor-related protein [61], these results are consistent with the possibility that the promotion of neurite extension by apoE-enriched β-VLDL is mediated through the LDL receptor-related protein. Further studies by Nathan and colleagues [35] utilizing the same in vitro system showed that human apoE3-enriched β-VLDL had similar effects as rabbit apoE-enriched β-VLDL in stimulating neurite extension, while conversely, human apoE4-enriched β-VLDL decreased neurite extension.

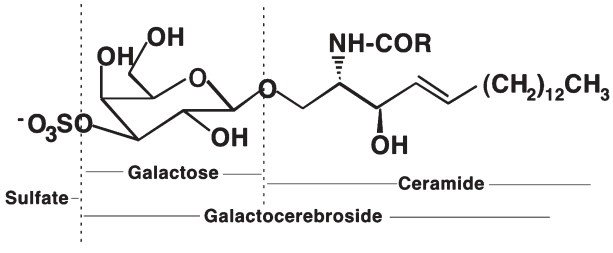
On the basis of protein mass, apoE is the predominant apolipoprotein found in the CSF that lacks apoB, another major cholesterol transport protein found in plasma [27]. Thus, it has long been postulated that apoE is critical for lipid transport and cholesterol homeostasis within the CNS, and many investigators have examined the effects of apoE or apoE isoforms on CNS lipid metabolism. However, despite these studies, the precise role of apoE in modulating lipid homeostasis in the CNS has not been thoroughly resolved. Therefore, the remainder of this review will attempt to explore whether apoE or apoE isoforms influence lipid content in the CNS. Recent results regarding the influence of apoE upon lipid homeostasis will be summarized with respect to apoE-mediated modulation of sulfatide content, cholesterol mass content and phospholipid molecular species in subcellular fractions in the CNS. The potential effects of these alterations on neurobiology will be also discussed.

Modulation of sulfatide metabolism by apoE

ApoE may play an important role in lipid metabolism and trafficking in the CNS where lipids including phospholipids, sphingolipids and cholesterol are very enriched and moreover, many kinds of specialized lipids such as cerebrosides, sulfatides and plasmalogens predominate. Recently, we have investigated the effects of apoE on lipid homeostasis using a lipidomics approach by exploiting the power inherited in electrospray ionization mass spectrometry (ESI/MS) [31]. By using this approach, we are able to profile and quantify each individual molecular species of all major and many minor lipid classes including phospholipids, sphingolipids, glycolipids, lipid metabolites, sterols and triacylglycerols (see [62, 63] for recent reviews). We determined the mass levels of individual molecular species of all classes of phospholipids, sphingomyelins, cerebrosides and sulfatides as well as the cholesterol in brain samples from human apoE3 and apoE4 transgenic mice and apoE knockout mice in comparison with those obtained from wild-type mice [31]. We demonstrated specific alterations in sulfatide mass and molecular species modulated by apoE. Sulfatides are a class of sulfated galactocerebrosides that only differ in the acyl constituents linked to the amino group of the sphingosine (scheme 1). Sulfatides are exclusively synthesized in oligodendrocytes in the CNS [64, 65].

Specifically, the mass content of sulfatides in hippocampus, cortex and cerebellum of apoE–/– mice were higher by 61, 114 and 7 mol%, respectively, relative to those found in apoE+/+ mice at the same age (12 months). Similar results were observed with other brain regions examined, such as thalamus, striatum and septum [31]. The levels of sulfatide mass in the CNS were modulated in an apoE isoform-dependent manner, resulting in mouse CNS sulfatide contents in decreasing order: apoE knockout > wild type > apoE3 transgenic > apoE4 transgenic [31]. It was also found that alterations of sulfatide content in apoE transgenic or knockout mice relative to wild-type mice are age dependent [31]. Accordingly, these experimental results demonstrate the isoform-specific regulation of sulfatide mass levels by apoE. The fact that human apoE3 and apoE4 transgenic mice, which express apoE selectively in brain, have lower levels of sulfatide than wild-type mice demonstrates that the regulation of brain sulfatide levels occurs through apoE produced in the brain. Importantly, in contrast to sulfatides, the mass levels of all major classes of phospholipids and sphingolipids in the CNS are not affected by mouse apoE and/or human apoE isoforms [31].

To elucidate the mechanism(s) by which apoE mediates sulfatide mass levels in the CNS, multiple assays to test the direct and/or indirect interactions between sulfatides and apoE were conducted [31]. It was found that sulfatides precisely colocalized with HDL-like lipoproteins present in human CSF. Because the two most abundant



Scheme 1. The structure of sulfatide. R represents aliphatic chain with or without a hydroxy group at α-carbon position.

apolipoproteins produced in the CNS are apoE and apoJ [7, 28], further experiments were performed to identify the specific carrier(s) of sulfatides in human CSF by immunoprecipitation, followed by ESI/MS quantitation of sulfatide content, and demonstrated that sulfatide molecules are specifically associated with apoE-containing HDL-like lipoproteins in CSF. These results also imply that sulfatides are transported by apoE-associated lipoproteins in brain interstitial fluid and CSF and endocytotic recycling of apoE particles through LDL receptor or its family members [66].

We also examined whether the sulfatide composition of both astrocyte-secreted apoE particles and human CSF are dependent upon the presence of specific apoE isoforms [31]. Purified astrocyte-secreted lipoproteins containing either human apoE3 or human apoE4 were prepared from cultured astrocytes. Following quantification of sulfatide content by ESI/MS, it was found that the sulfatide content of these particles was low, although apoE4 particles contained more sulfatide mass than that found in apoE3 particles (0.73 ± 0.05 vs. 0.56 ± 0.02 nmol/mg apoE, respectively). The sulfatide content in CSF samples from age-matched cognitively normal human subjects was analyzed by ESI/MS techniques. The sulfatide mass (nmol/mg apoE) in apoE3/E3 homozygous subjects was 46.4 ± 3.3, whereas sulfatide levels were significantly higher in subjects with 1 or 2 alleles of apoE4 (53.7 ± 4.6, $P < 0.01$). It should be noted that the sulfatide content in CSF samples from subjects who were either heterozygous or homozygous for *APOE4* (3/4 or 4/4) were indistinguishable in the study. Thus, human CSF contains significant amounts of sulfatide, and the mass level of sulfatide in human CSF is apoE isoform dependent. Because sulfatides are almost exclusively synthesized by oligodendrocytes in the central nervous system and are present predominantly in the myelin sheath surrounding axons [65], these results also suggest that apoE particles mainly acquire sulfatides in the CNS after secretion from astrocytes.

Sulfatides play critical roles in various biological processes such as the regulation of cell growth, protein trafficking, signal transduction, cell adhesion, neuronal plasticity and cell morphogenesis (see [65, 67, 68] for reviews). For example, accumulation of sulfatides, due to a sulfatidase deficiency, is responsible for metachromatic leukodystrophy [69]. Mice deficient in sulfatide and galactosylcerebrosides, generated by knocking out a ceramide galactosyltransferase, generally die by 3 months of age and demonstrate many abnormalities including abnormal axonal function, dysmyelinosis and loss of axonal conduction velocity [70–73].

Thus, the implications of the role of apoE-mediated sulfatide mass alteration in neurobiology are significant. Although the apoE $\varepsilon 4$ allele is the only known genetic risk factor for late-onset Alzheimer's disease (AD) [43, 74, 75], the mechanism(s) by which apoE participates in AD pathogenesis is not clear. Moreover, we recently demonstrated that substantial sulfatide deficiency (up to 92%) occurs in both AD white and gray matter in all examined brain regions, including frontal, temporal, parietal and cerebellum at the earliest clinically recognizable stage of the disease (i.e. mild cognitive impairment due to Alzheimer's type dementia) [76]. It was found that in addition to the sulfatide deficiency, which occurs in postmortem brain tissues of AD subjects, substantially depleted CSF sulfatides were manifest in subjects with incipient dementia of Alzheimer's type [77]. Although the cause(s) of this deficiency remains unclear, a linkage between apoE function and sulfatide deficiency in the development of AD is obvious.

Zarepesi and colleagues [57] observed that apoE4 appears to influence the age of onset of Parkinson's disease. Intriguingly, we have recently found that sulfatides substantially accumulated in subjects with Parkinson's disease (PD) [78]. It is well known that the accumulation of sulfatides is responsible for metachromatic leukodystrophy. Whether gradual accumulation of sulfatides in neuronal cells is associated with the pathogenesis of PD remains to be elucidated. Very recently, Tarkowski and colleagues [79] demonstrated alterations in sulfatide mass in CSF of subjects with vascular dementia, suggesting that apoE-mediated sulfatide metabolism might be involved in this disease too.

Although available evidence supports the conclusion that sulfatides are only synthesized in oligodendrocytes [64, 65], the presence of sulfatides in neurons and astrocytes (e.g. [80, 81]) suggest the existence of transport and endocytotic pathways for sulfatide-associated apoE particles and the requirement of sulfatides in some of the neuronal functions such as neuronal regeneration. Following this line, it can be speculated that sulfatides may play a role in neuronal regeneration directly through uptake of sulfatide in neurons through endocytotic pathways or indirectly through myelin sheath function. Conversely, accumulation of sulfatides caused by deficient lysosomal enzyme sulfatidase activity in neuronal cells results in metachromatic leukodystrophy, in which there is encephalopathy, deposits of metachromatic granules in both CNS and PNS, and degeneration of CNS myelin [69]. This evidence further supports the importance of sulfatide metabolism through the endocytotic pathway of apoE particles and suggests the critical role of apoE-mediated sulfatide metabolism in normal neuronal homeostasis.

Mahley and Rall [33] discussed the nontraditional roles of apoE in immunoregulation and in susceptibility to infectious disease. Intriguingly, numerous studies have shown that sulfatides are ligands for a family of receptor proteins, called selectins, located on activated vascular endothelial cells, leukocytes and activated platelets [82–88]. While the relationship between the role of apoE

in immunoregulation and sulfatide as a potential ligand for selectins is unclear, it has been shown that sulfatides are potent anti-inflammatory agents [89]. As such, sulfatides in apoE particles may serve as anti-inflammatory immunomodulatory molecules. It is also possible, particularly in the case of multiple sclerosis and possibly stroke, that apoE is in some way regulating CNS inflammatory processes such as cytokine production, as has been seen in some models (e.g. [90]).

ApoE4 lipoproteins can carry more sulfatides than their apoE3 counterparts in the CNS [31], and it is probable that sulfatides play important roles in anti-inflammation and in protection against certain infectious diseases [89]. Such evidence supports an evolutionary theory proposed by Finch and Sapolsky [91] that $\varepsilon 4$ could have survived because it might be advantageous early in life but disadvantageous later in life. Therefore, the frequency of apoE4 is higher in certain areas where protection against infectious disease remains a priority, such as in the African subcontinent and in certain other isolated populations (e.g. in Papua New Guinea). Clearly, apoE-associated sulfatides and their roles in neurobiology, inflammation and infectious disease need to be further explored.

Influence of apoE phenotype on cholesterol mass content in the CNS

Numerous studies have demonstrated the role of apoE in the normal maintenance and transport of plasma lipids in an isoform-dependent manner (see [14, 33] for reviews). As a particular example, Dallongeville and colleagues [92] did a meta-analysis to illustrate the association between the apoE phenotype and lipid levels in the combined data of published studies between 1983 and 1991. A total of 14799 individuals from 45 population samples from 17 different countries were included in the analysis. In the analysis, they confirmed previous observations that individuals carrying the *APOE*2 and *APOE*4 alleles have lower and higher levels of plasma cholesterol, respectively, than those with the homozygous *APOE*3 genotype. They found that a similar relationship is present within the different populations, suggesting that the role of the apoE polymorphism in modulating mass levels of plasma cholesterol is homogeneous within different ethnic groups or metabolic situations [92]. However, different investigators might observe the effects of the apoE phenotypes on the modulation of plasma cholesterol levels to varying degrees, because the magnitude of the effects of various apoE alleles could differ among populations and individual health states [93, 94].

It has been demonstrated that apoE is a major apolipoprotein constituent of CSF (~5 μg/ml), where it is associated with HDL-like particles [29, 95]. Multiple investigators have undertaken studies to determine the relationship between the composition of CSF lipoproteins and the apoE genotype [96–103]. The determination of this relationship is necessary because CSF apoE could potentially be produced either in the CNS or by the liver and reach the CNS by diffusion across the blood-brain barrier, as has been observed for apoAI (~4 μg/ml) in CSF [103]. These studies demonstrated, however, that there is no correlation of apoE and associated lipids between CSF and serum, suggesting that apoE lipoproteins are unable to cross the blood-brain barrier and that the concentrations of CSF apoE are regulated within the CNS.

One of the first questions to be addressed was whether the apoE genotype affects the mass content of CSF cholesterol, as has been found for serum apoE. Yamauchi and colleagues [101] determined the mass levels of CSF apoE and CSF total cholesterol. After analyzing the relationship between the mass levels of CSF cholesterol and the apoE phenotype, they found that although no significant differences in total CSF cholesterol with respect to sex or age were present, the cholesterol concentrations in individuals with the apoE phenotype E4/E3 (1.34 ± 0.723 mg/l, mean ± SD, $n = 8$) were significantly lower ($P = 0.004$) than in those with the apoE phenotype E3/E3 (2.78 ± 1.295 mg/l, $n = 38$) and E3/E2 (2.98 ± 2.391, $n = 6$).

However, in a later study, Fagan and colleagues [103] for the first time employed stringent selection criteria for the identification of cognitively normal, healthy, elderly subjects. Samples (both CSF and plasma) were collected in a fasted state; therefore, it is possible to directly compare plasma and CSF lipoproteins from different subjects. They determined the mass levels of both CSF and serum apoE and total cholesterol with other biochemical parameters. They found (i) no correlation between CSF and serum in the mass levels of apoE or cholesterol in cognitively normal subjects, suggesting local regulation of brain lipoprotein metabolism; (ii) a significant correlation between the mass levels of CSF apoE and CSF total cholesterol, suggesting the association of CSF cholesterol with CSF apoE; and (iii) a strong correlation between the mass levels of CSF apoAI and serum apoAI, indicating a plasma source of CSF apoAI. In contrast to Yamauchi and colleagues [101], Fagan and colleagues [103] did not observe effects of differences in apoE phenotype on the CSF cholesterol levels when comparing E3/E3 subjects ($n = 13$, 2.40 ± 0.2 mg/l) with any E4 (i.e. E3/E4 and E4/E4) subjects ($n = 12$, 2.47 ± 0.19 mg/l). It is interesting to mention that Fagan and colleagues did observe a much lower CSF cholesterol mass level from two E4/E4 subjects than other groups. Because this observation was only from two cases, no conclusion was made by the authors. Definitive answers to questions regarding the relationship between apoE genotype and CSF cholesterol mass levels may need studies utilizing larger number of subjects, as has been done for plasma apoE [92]. How-

ever, selection of well-controlled individuals is undoubtedly important for such studies.

A logical second question in this context is whether apoE actually modulates cholesterol mass levels in brain tissues. Very recently, we determined the mass levels of cholesterol in different brain regions, including cerebral cortex, cerebellum and hippocampus, from wild-type and various apoE transgenic mice, including apoE knockout, human apoE3 transgenic and human apoE4 transgenic at 4–24 months of age [31]. We found that the cholesterol mass levels were very consistent in each specific brain region, such as cortex, cerebellum or hippocampus, between wild-type and various apoE transgenic mice, whereas differences in cholesterol mass levels between the brain regions were present within the same type of mice. These results are consistent with multiple recent studies on cholesterol mass content in apoE knockout mice [30, 104–106]. In another study, Kirsch and colleagues [106] examined the effects of dietary cholesterol on the mass levels of cholesterol in the brain. They fed young Albino Wistar rats a diet containing 2% cholesterol for at least 6 months and found no effects on brain cholesterol levels of synaptosomal plasma membranes of the cortex relative to the control animals, whereas plasma and liver cholesterol significantly increased, suggesting the differential role of apoE in cholesterol homeostasis in plasma and in the CNS. Following this line, however, a number of other studies using AD model (e.g. APP transgenic) mice demonstrated that brain cholesterol content was significantly increased when the mice were fed with a high-fat/high-cholesterol diet for several weeks [107–109]. Because brain cholesterol mass levels in wild-type mice were not altered after being fed a high-cholesterol diet [106], the reason behind the higher brain cholesterol levels in AD model animals after being fed a high-cholesterol diet is important and needs to be further investigated.

During the assessment of lipid alterations in subcellular membranes induced by apoE deficiency, Tietz and colleagues [110] found that the cholesterol levels of microsomal membranes are affected by apoE. However, they did not observe changes in cholesterol mass levels within the synaptosomal membranes due to the absence of apoE. Although they did not observe alterations in cholesterol mass levels in brain tissues due to apoE depletion, Hayashi and colleagues [105] did find that the cholesterol content in the exofacial leaflet of synaptic plasma membranes is altered in human apoE4 knockin mice. Moreover, recent studies by Pfrieger and colleagues [111, 112] demonstrated that CNS synaptogenesis required a large amount of cholesterol, which was derived from glial cells through the transport of apoE-containing lipoproteins. Other studies on the metabolism of cholesterol in the CNS have recently been reviewed [112, 113]. Furthermore, Michikawa and colleagues [114, 115] showed that exogenously added or astrocyte-secreted apoE could promote cholesterol release or transport, respectively, in an apoE-isoform-dependent fashion. Therefore, although cholesterol mass levels are not affected by the apoE genotype or even by apoE depletion, the possibility that cholesterol mass levels and trafficking of specialized subcellular compartments are altered isoform-specifically by apoE cannot be excluded. Further studies in this area are necessary.

Collectively, although apoE plays an important role in plasma cholesterol trafficking and metabolism, and it has been assumed that apoE may serve a similar function in the CNS, available data derived from CSF and brain tissues do not support such a hypothesis. On the other hand, substantial evidence indicates that the role of apoE in neurobiology may be related to cholesterol homeostasis ([23], also see previous discussion), and it has been observed that alterations of cholesterol mass content in some subcellular membranes are induced by apoE. Therefore, future studies on the effects of apoE on cholesterol trafficking and metabolism may need to focus on subcellular membrane compartments, membrane leaflets and/or membrane microdomains such as caveolae and lipid rafts.

ApoE-mediated alterations in phospholipid molecular species containing polyunsaturated acyl chains in specialized membrane compartments

Membrane phospholipids are classified into different classes based on the nature of the polar head groups [63]. Three major subclasses are present in each phospholipid class based on the covalent linkage of the aliphatic chain at the *sn*-1 position of the glycerol backbone. Furthermore, individual phospholipid molecular species differ in the constituents of two aliphatic chains due to the chain length as well as the number and location of double bonds [63]. Whether apoE plays a role in the trafficking and metabolism of these complex phospholipids in the CNS is an interesting topic. Many research groups, including the author's laboratory (e.g. [30, 31, 116, 117]), have exploited a variety of techniques to address this issue.

Montine and colleagues [116] reported significantly lower total brain phospholipid mass ($P < 0.05$) in apoE-deficient mice than that in control mice. However, by employing a lipidomic approach [62, 63], we quantitatively analyzed global lipids, including each individual molecular species of all phospholipid classes in different mouse brain regions such as cerebral cortex, cerebellum and hippocampus [31]. We did not observe any alterations in either the mass levels of total phospholipids or the profiles of individual molecular species in apoE knockout, human apoE3 transgenic and apoE4 trans-

genic mice in comparison with wild-type controls. These results are comparable with a study on brain membrane phospholipids from apoE knockout mice [30]. The differences in phospholipid mass content as reported by Montine and colleagues [116] may have resulted from cross-contamination of isolated brain tissues, since a large amount of tissue is required when traditional analytical methods are employed. In contrast, the purity of tissue samples can be confirmed by examination of the lipid profiles using an ESI/MS lipidomic approach, as previously described [118].

Although no influence of apoE on phospholipid homeostasis in brain tissues and major membrane fractions has been observed, changes in both the mass levels of total phospholipids and the profiles of individual molecular species in some specialized membrane compartments are present. For example, Lomnitski and colleagues [30] analyzed brain phospholipids employing traditional techniques such as thin-layer chromatography and gas liquid chromatography. They found that the total mass levels of phosphatidylcholine (PtdCho) in brain microsomal membrane fraction are significantly lower in apoE-deficient mice ($P < 0.05$) compared with those in the control samples. This change would appear to be very specific, since neither the mass levels of ethanolamine glycerophospholipid (PE) in brain microsomal membrane nor the mass levels of PtdCho and PE in the membrane fraction containing plasma and mitochondrial membranes were altered in apoE-deficient mice relative to the controls [30]. Furthermore, Lomnitski and colleagues found the effects of apoE on fatty acid composition of phospholipids were different from the effects of apoE on phospholipid mass. No changes were observed in the fatty acid composition of PtdCho between brain microsomal membranes prepared from apoE-deficient in comparison with wild-type mice. In contrast, analyses of the fatty acid composition of PE and phosphatidylserine (PtdSer) revealed apoE-dependent alterations. Specifically, PE contained lower while PtdSer contained higher ratios of polyunsaturated to saturated fatty acids, respectively, in apoE-deficient mice compared with those in controls. Again, these effects of apoE on fatty acid composition were not found in the subcellular membrane fractions containing plasma membranes or mitochondrial membranes. These findings indicate that the effects of apoE on phospholipid trafficking and metabolism are specific and intracellular compartment dependent, suggesting a role of apoE in mediating multiple differential neuronal functions.

Igbavboa and colleagues [117] identified and quantitated phospholipid molecular species by reverse-phase high-performance liquid chromatography (HPLC)/electrospray ionization mass spectrometry and determined the ability of apoE to alter specific phospholipid molecular species in neuronal membranes (i.e. synaptic plasma membranes). Although no obvious differences in mass of different phospholipid classes were present in membranes prepared from apoE-deficient in comparison with wild-type mice, there were substantial differences in composition of phospholipid molecular species. Specifically, the predominant molecular species in choline, ethanolamine and serine glycerophospholipids contained polyunsaturated acyl chains at the *sn*-2 position, while in synaptic plasma membranes prepared from apoE-deficient mice were significantly decreased in their content of polyunsaturated fatty acids. In contrast, molecular species containing two polyunsaturated fatty acids, such as 22:4/22:6 and 22:6/22:6 in PtdCho, PE and PtdSer, accumulated in synaptic plasma membranes of apoE-deficient mice relative to control mice [117]. These membranes, which were isolated from the terminals of neurons, play critical roles in numerous neuronal functions and are enriched with a variety of receptors, ion channels and enzymes. Igbavboa and colleagues [117] found that a substantial number of di-polyunsaturated phospholipid molecular species were present in synaptic plasma membranes, suggesting the requirement of a highly fluid environment for synaptic terminals and a new role for apoE in regulating polyunsaturated phospholipid molecular species trafficking in neuronal membranes.

Conclusion

Many studies have now shown that apoE is enriched in glial cells, particularly astrocytes [17, 119, 120]. Although neurons do not express apoE themselves [21, 22, 121, 122], they do posses receptors to endocytose apoE, and the presence of apoE in the internal neural vesicles has also been demonstrated [123]. Therefore, apoE can directly modulate sulfatide mass content in the CNS through the same metabolic pathways that regulate levels of apoE-containing CNS lipoproteins (i.e. through endocytosis and transporting to CSF), since sulfatides are tightly associated with apoE-containing particles [31]. Available data demonstrate that apoE does not affect the mass of cholesterol in both CSF and brain tissues. Similarly, both the total mass levels and the molecular species profiles of phospholipids in brain tissues are not altered by apoE. However, multiple lines of evidence indicate that the mass of cholesterol and phospholipids and/or the molecular species profiles of phospholipids are altered in specialized membrane compartments (e.g. synaptic plasma membranes) or membrane leaflets. Collectively, it can be concluded that apoE mediates sulfatide trafficking and metabolism in the CNS. Moreover, although apoE does not apparently affect cholesterol mass content and phospholipid mass levels and composition in the CNS overall, apoE has been demonstrated to modulate cholesterol and phospholipid homeostasis in some specialized subcellular membrane compartments, such as synaptic

plasma membranes. Because available data on the effects of apoE on lipid homeostasis are still quite limited, further studies in the area, particularly in the influence of apoE on membrane compartment and/or microdomain composition or trafficking, are certainly important. Clearly, a thorough understanding of the role of apoE in lipid metabolism in the CNS will provide important new insights into the mechanism(s) underlying the roles of apoE in neurobiology.

Acknowledgement. This work was supported by NIH grant PO1HL57278. The author is the 2003 Memory Ride Prize recipient. The author is grateful to Drs Christopher M. Jenkins and Anne M. Fagan for their comments.

1 Mackness M. I. and Durrington P. N. (1992) Lipoprotein separation and analysis for clinical studies. In: Lipoprotein Analysis: A Practical Approach, pp. 1–42, Converse C. A. and Skinner E. R. (eds), Oxford University Press, New York
2 Brown M. S. and Goldstein J. L. (1986) A receptor-mediated pathway for cholesterol homeostasis. Science **232:** 34–47
3 Fredenrich A. and Bayer P. (2003) Reverse cholesterol transport, high density lipoproteins and HDL cholesterol: recent data. Diabetes Metab. **29:** 201–205
4 Rader D. J. (2003) Regulation of reverse cholesterol transport and clinical implications. Am. J. Cardiol. **92:** 42J–49J
5 Wang M. and Briggs M. R. (2004) HDL: The metabolism, function and therapeutic importance. Chem. Rev. **104:** 119–137
6 Ladu M. J., Reardon C., van Eldik L., Fagan A. M., Bu G., Holtzman D. M. et al. (2000) Lipoproteins in the central nervous system. Ann. N. Y. Acad. Sci. **903:** 167–175
7 Koch S., Donarski N., Goetze K., Kreckel M., Stuerenburg H.-J., Buhmann C. et al. (2001) Characterization of four lipoprotein classes in human cerebrospinal fluid. J. Lipid Res. **42:** 1143–1151
8 Rebeck G. W., Reiter J. S., Strickland D. K. and Hyman B. T. (1993) Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. Neuron **11:** 575–580
9 Li W. H., Tanimura M., Luo C. C., Datta S. and Chan L. (1988) The apolipoprotein multigene family: biosynthesis, structure, structure-function relationships and evolution. J. Lipid Res. **29:** 245–271
10 Danik M., Champagne D., Petit-Turcotte C., Beffert U. and Poirier J. (1999) Brain lipoprotein metabolism and its relation to neurodegenerative disease. Crit. Rev. Neurobiol. **13:** 357–407
11 de Knijff P., van den Maagdenberg A. M. J. M., Frants R. R. and Havekes L. M. (1994) Genetic heterogeneity of apolipoprotein E and its influence on plasma lipid and lipoprotein levels. Hum. Mutat. **4:** 178–194
12 Nickerson D. A., Taylor S. L., Fullerton S. M., Weiss K. M., Clark A. G., Stengard J. H. et al. (2000) Sequence diversity and large-scale typing of SNPs in the human apolipoprotein E gene. Genome Res. **10:** 1532–1545
13 Laws S. M., Hone E., Gandy S. and Martins R. N. (2003) Expanding the association between the APOE gene and the risk of Alzheimer's disease: possible roles for APOE promoter polymorphisms and alterations in APOE transcription. J. Neurochem. **84:** 1215–1236
14 Mahley R. W. (1988) Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. Science **240:** 622–630
15 Smith J. (2002) Apolipoproteins and aging: emerging mechanisms. Ageing Res. Rev. **1:** 345–365
16 Lin C. T., Xu Y., Wu J. Y. and Chan L. (1986) Immunoreactive apolipoprotein E is a widely distributed cellular protein. Immunohistochemical localization of apolipoprotein E in baboon tissues. J. Clin. Invest. **78:** 947–958
17 Elshourbagy N. A., Liao W. S., Mahley R. W. and Taylor J. M. (1985) Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. Proc. Natl. Acad. Sci. USA **82:** 203–207
18 Srivastava R. A. K., Bhasin N. and Srivastava N. (1996) Apolipoprotein E gene expression in various tissues of mouse and regulation by estrogen. Biochem. Mol. Biol. Int. **38:** 91–101
19 Mahley R. W., Innerarity T. L., Weisgraber K. H. and Oh S. Y. (1979) Altered metabolism (in vivo and in vitro) of plasma lipoproteins after selective chemical modification of lysine residues of the apoproteins. J. Clin. Invest. **64:** 743–750
20 Linton M. F., Gish R., Hul S. T., Butler E., Esquivel C., Bry W. I. et al. (1991) Phenotypes of apolipoprotein B and apolipoprotein E after liver transplantation. J. Clin. Invest. **88:** 270–281
21 Boyles J. K., Pitas R. E., Wilson E., Mahley R. W. and Taylor J. M. (1985) Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. J. Clin. Invest. **76:** 1501–1513
22 Pitas R. E., Boyles J. K., Lee S. H., Foss D. and Mahley R. W. (1987) Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E-containing lipoproteins. Biochim. Biophys. Acta **917:** 148–161
23 Pfrieger F. W. (2003) Outsourcing in the brain: do neurons depend on cholesterol delivery by astrocytes? Bioessays **25:** 72–78
24 Weisgraber K. H. (1994) Apolipoprotein E: Structure-function relationships. Adv. Protein Chem. **45:** 249–302
25 Mahley R. W. and Rall S. C. Jr (1995) Type III hyperlipoproteinemia: the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: The Metabolic and Molecular Bases of Inherited Disease, 7th edn, pp. 1953–1980, Scriver C. R., Beaudet A. L., Sly W. S. and Valle D. (eds), McGraw-Hill, New York
26 Mahley R. W., Huang Y. and Rall S. C. Jr (1999) Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia): questions, quandaries and paradoxes. J. Lipid Res. **40:** 1933–1949
27 Merched A., Blain H., Visvikis S., Herbeth B., Jeandel C. and Siest G. (1997) Cerebrospinal fluid apolipoprotein E level is increased in late-onset Alzheimer's disease. J. Neurol. Sci. **145:** 33–39
28 DeMattos R. B., Brendza R. P., Heuser J. E, Kierson M., Cirrito J. R., Fryer J. et al. (2001) Purification and characterization of astrocyte-secreted apolipoprotein E and J-containing lipoproteins from wild-type and human apoE transgenic mice. Neurochem. Int. **39:** 415–425
29 Pitas R. E., Boyles J. K., Lee S. H., Hui D. and Weisgraber K. H. (1987) Lipoproteins and their receptors in the central nervous system. Characterization of the lipoproteins in cerebrospinal fluid and identification of apolipoprotein B, E (LDL) receptors in the brain. J. Biol. Chem. **262:** 14352–14360
30 Lomnistski L., Oron L., Sklan D. and Michaelson D. M. (1999) Distinct alterations in phospholipid metabolism in brains of apolipoprotein E-deficient mice. J. Neurosci. Res. **58:** 586–592
31 Han X., Cheng H., Fryer D. J., Fagan A. M. and Holtzman D. M. (2003) Novel role for apolipoprotein E in the central nervous system: modulation of sulfatide content. J. Biol. Chem. **278:** 8043–8051
32 Horsburgh K., McCarron M. O., White F. and Nicoll J. A. R. (2000) The role of apolipoprotein E in Alzheimer's disease,

acute brain injury and cerebrovascular disease: evidence of common mechanisms and utility of animal models. Neurobiol. Aging **21:** 245–255
33 Mahley R. W. and Rall S. C. Jr (2000) Apolipoprotein E: far more than a lipid transport protein. Annu. Rev. Genom. Hum. Genet. **1:** 507–537
34 Ignatius M. J., Shooter E. M., Pitas R. E. and Mahley R. W. (1987) Lipoprotein uptake by neuronal growth cones in vitro. Science **236:** 959–962
35 Nathan B. P., Bellosta S., Sanan D. A., Weisgraber K. H., Mahley R. W. and Pitas R. E. (1994) Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. Science **264:** 850–852
36 Holtzman D. M., Pitas R. E., Kilbridge J., Nathan B., Mahley R. W., Bu G. et al. (1995) Low density lipoprotein receptor-related protein mediates apolipoprotein E-dependent neurite outgrowth in a central nervous system-derived neuronal cell line. Proc. Natl. Acad. Sci. USA **92:** 9480–9484
37 Buttini M., Orth M., Bellosta S., Akeefe H., Pitas R. E., Wyss-Coray T. et al. (1999) Expression of human apolipoprotein E3 or E4 in the brains of Apoe–/– mice: isoform-specific effects on neurodegeneration. J. Neurosci. **19:** 4867–4880
38 Boyles J. K., Notterpek L. M. and Anderson L. J. (1990) Accumulation of apolipoproteins in the regenerating and remyelinating mammalian peripheral nerve. Identification of apolipoprotein D, apolipoprotein A-IV, apolipoprotein E and apolipoprotein A-I. J. Biol. Chem. **265:** 17805–17815
39 Masliah E., Mallory M., Ge N., Alford M., Veinbergs I. and Roses A. D. (1995) Neurodegeneration in the central nervous system of apoE-deficient mice. Exp. Neurol. **136:** 107–122
40 Poirier J., Baccichet A., Dea D. and Gauthier S. (1993) Cholesterol synthesis and lipoprotein reuptake during synaptic remodeling in hippocampus in adult rats. Neuroscience **55:** 81–90
41 de Chaves E. I. P., Vance D. E., Campenot R. B., Kiss R. S., and Vance J. E. (2000) Uptake of lipoproteins for axonal growth of sympathetic neurons. J. Biol. Chem. **275:** 19883–19890
42 Popko B., Goodrum J. F., Bouldin T. W., Zhang S. H. and Maeda N. (1993) Nerve regeneration occurs in the absence of apolipoprotein E in mice. J. Neurochem. **60:** 1155–1158
43 Strittmatter W. J. and Roses A. D. (1996) Apolipoprotein E and Alzheimer disease. Annu. Rev. Neurosci. **19:** 53–77
44 Weisgraber K. H. and Mahley R. W. (1996) Human apolipoprotein E: the Alzheimer's disease connection. FASEB J. **10:** 1485–1494
45 Wisniewski T. and Frangione B. (1992) Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. Neurosci. Lett. **135:** 235–238
46 Strittmatter W. J., Weisgraber K. H., Huang D. Y., Dong L., Salvesen G. S., Pericak-Vance M. et al. (1993) Binding of human apolipoprotein E to synthetic amyloid β peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc. Natl. Acad. Sci. USA **90:** 8098–8102
47 Bales K. R., Verina T., Dodel R. C., Du Y., Altstiel L., Bender M. et al. (1997) Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. Nat. Genet. **17:** 263–264
48 Holtzman D. M., Bales K. R., Tenkova T., Fagan A. M., Parsadanian M., Sartorius L. J. et al. (2000) Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA **97:** 2892–2897
49 DeMattos R. B., Cirrito J. R., Parsadanian M., May P. C., O'Dell M. A., Taylor J. W. et al. (2004) ApoE and clusterin cooperatively suppress Aβ levels and deposition: evidence that ApoE regulates extracellular Aβ metabolism in vivo. Neuron **41:** 193–202
50 Nicoll J. A., Roberts G. W. and Graham D. I. (1995) Apolipoprotein E epsilon 4 allele is associated with deposition of amyloid beta-protein following head injury. Nat. Med. **1:** 135–137
51 Teasdale G. M., Nicoll J. A., Murray G. and Fiddes M. (1997) Association of apolipoprotein E polymorphism with outcome after head injury. Lancet **350:** 1069–1071
52 Friedman G., Froom P., Sazbon L., Grinblatt I., Shochina M., Tsenter J. et al. (1999) Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. Neurology **52:** 244–248
53 Nathoo N., Chetty R., van Dellen J. R. and Barnett G. H. (2003) Genetic vulnerability following traumatic brain injury: the role of apolipoprotein E. Mol. Pathol. **56:** 132–136
54 Alberts M. J., Graffagnino C., McClenny C., DeLong D., Strittmatter W., Saunders A. M. et al. (1995) ApoE genotype and survival from intracerebral haemorrhage. Lancet **346:** 575
55 McCarron M. O., Hoffmann K. L., DeLong D. M., Gray L., Saunders A. M. et al. (1999) Intracerebral hemorrhage outcome: apolipoprotein E genotype, hematoma and edema volumes. Neurology **53:** 2176–2179
56 Slooter A. J., Tang M. X., van Duijn C. M., Stern Y., Ott A., Bell K. et al. (1997) Apolipoprotein E epsilon4 and the risk of dementia with stroke. A population-based investigation. J. Am. Med. Assoc. **277:** 818–821
57 Zarepesi S., Kaye J., Camicioli R., Grimslid H., Oken B., Litt M. et al. (1997) Modulation of the age at onset of Parkinson's disease by apolipoprotein E genotype. Ann. Neurol. **42:** 655–658
58 Boyles J. K., Zoellner C. D., Anderson L. J., Kosik L. M., Pitas R. E., Weisgraber K. H. et al. (1989) A role for apolipoprotein E, apolipoprotein A-I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. J. Clin. Invest. **83:** 1015–1031
59 Goodrum J. F. (1991) Cholesterol from degenerating nerve myelin becomes associated with lipoproteins containing apolipoprotein E. J. Neurochem. **56:** 2082–2086
60 Handelmann G. E., Boyles J. K., Weisgraber K. H., Mahley R. W. and Pitas R. E. (1992) Effects of apolipoprotein E, beta-very low density lipoproteins and cholesterol on the extension of neurites by rabbit dorsal root ganglion neurons in vitro. J. Lipid Res. **33:** 1677–1688
61 Kowal R. C., Herz J., Goldstein J. L., Esser V. and Brown M. S. (1989) Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. Proc. Natl. Acad. Sci. USA **86:** 5810–5814
62 Han X. and Gross R. W. (2003) Global analysis of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. J. Lipid Res. **44:** 1071–1079
63 Han X. and Gross R. W. (2004) Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of the cellular lipidomes directly from crude extracts of biological samples. Mass Spec. Rev., in press
64 Svennerholm L., Bostrom K., Fredman P., Jungbjer B., Mansson J. E. and Rynmark B. M. (1992) Membrane lipids of human peripheral nerve and spinal cord. Biochim. Biophys. Acta **1128:** 1–7
65 Vos J. P., Lopes-Cardozo M. and Gadella B. M. (1994) Metabolic and functional aspects of sulfogalactolipids. Biochim. Biophys. Acta **1211:** 125–149
66 Krieger M. and Herz J. (1994) Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). Ann. Rev. Biochem. **63:** 601–637
67 Merrill Jr. A. H., Schmelz E.-M., Dillehay D. L., Spiegel S., Shayman J. A., Schroeder J. J. et al. (1997) Sphingolipids-the enigmatic lipid class: biochemistry, physiology and pathophysiology. Toxicol. Appl. Pharmacol. **142:** 208–225
68 Ishizuka I. (1997) Chemistry and functional distribution of sulfoglycolipids. Prog. Lipid Res. **36:** 245–319

69 von Figura K., Gieselmann V. and Jaeken J. (2001) Metachromatic leukodystrophy: Lysosomal disorders. In: The Metabolic and Molecular Bases of Inherited Diseases, Scriver C. R., Beaudet A. L., Sly W. S. and Valle D. (eds), 8th edn, pp. 3695–3724, McGraw-Hill, New York

70 Bosio A., Binczek E. and Stoffel W. (1996) Functional breakdown of the lipid bilayer of the myelin membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis. Proc. Natl. Acad. Sci. USA **93:** 13280–13285

71 Coetzee T., Fujita N., Dupree J., Shi R., Blight A., Suzuki K. et al. (1996) Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. Cell **86:** 209–219

72 Bosio A., Büssow H., Adam J. and Stoffel W. (1998) Galactosphingolipids and axono-glial interaction in myelin of the central nervous system. Cell Tissue Res. **292:** 199–210

73 Coetzee T., Suzuki K. and Popko B. (1998) New perspectives on the function of myelin galactolipids. Trends Neurosci. **21:** 126–130

74 Saunders A. M., Strittmatter W. J., Schmechel D., St. George-Hyslop P. H., Pericak-Vance M. A., Joo S. H. et al. (1993) Association of apolipoprotein E allele e4 with late-onset familial and sporadic Alzheimer's disease. Neurology **43:** 1467–1472

75 Poirier J., Davignon J., Bouthillier D., Kogan S., Bertrand P. and Gauthier S. (1993) Apolipoprotein E polymorphism and Alzheimer's disease. Lancet **342:** 697–699

76 Han X., Holtzman D. M., McKeel D. W. Jr, Kelley J. and Morris J. C. (2002) Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. J. Neurochem. **82:** 809–818

77 Han X., Fagan A. M., Cheng H., Morris J. C., Xiong C. and Holtzman D. M. (2003) Cerebrospinal fluid sulfatide is decreased in subjects with incipient dementia. Ann. Neurol. **54:** 115–119

78 Cheng H., Xu J., McKeel D. W. Jr and Han X. (2003) Specificity and potential mechanism of sulfatide deficiency in Alzheimer's disease: an electrospray ionization mass spectrometric study. Cell. Mol. Biol. **49:** 809–818

79 Tarkowski E., Tullberg M., Fredman P. and Wikkelsoe C. (2003) Correlation between intrathecal sulfatide and TNF-a levels in patients with vascular dementia. Dement. Geriat. Cogn. Disord. **15:** 207–211

80 Zalc B., Monge M., Dupouey P., Hauw J. J. and Baumann N. A. (1981) Immunohistochemical localization of galactosyl and sulfogalactosyl ceramide in the brain of the 30-day-old mouse. Brain Res. **211:** 341–354

81 Berntson Z., Hansson E., Ronnback L. and Fredman P. (1998) Intracellular sulfatide expression in a subpopulation of astrocytes in primary cultures. J. Neurosci. Res. **52:** 559–568

82 Todderud G., Alford J., Millsap K. A., Aruffo A. and Tramposch K. M. (1992) PMN binding to P-selectin is inhibited by sulfatide. J. Leukocyte Biol. **52:** 85–88

83 Suzuki Y., Toda Y., Tamatani T., Watanabe T., Suzuki T., Nakao T. et al. (1993) Sulfated glycolipids are ligands for a lymphocyte homing receptor, L-selectin (LECAM-1), binding epitope in sulfated sugar chain. Biochem. Biophys. Res. Commun. **190:** 426–434

84 Bajorath J., Hollenbaugh D., King G., Harte W. Jr, Eustice D. C., Darveau R. P. et al. (1994) CD62/P-selectin binding sites for myeloid cells and sulfatides are overlapping. Biochemistry **33:** 1332–1339

85 Kajihara J., Ye G., Kato K. and Suzuki Y. (1995) Sulfatide, a specific sugar ligand for L-selectin, blocks CCl4-induced liver inflammation in rats. Biosci. Biotechnol. Biochem. **59:** 155–157

86 Mulligan M. S., Miyasaka M., Suzuki M., Kawashima H., Lizuka M., Hasegawa A. et al. (1995) Anti-inflammatory effects of sulfatides in selectin-dependent acute lung injury. Int. Immunol. **7:** 1107–1113

87 Bowman K. G., Cook B. N., de Graffenried C. L. and Bertozzi C. R. (2001) Biosynthesis of L-selectin ligands: sulfation of sialyl Lewis x-related oligosaccharides by a family of GlcNAc-6-sulfotransferases. Biochemistry **40:** 5382–5391

88 Merten M. and Thiagarajan P. (2001) Role for sulfatides in platelet aggregation. Circulation **104:** 2955–2960

89 Marinier A., Martel A., Banville J., Bachand C., Remillard R., Lapointe P. et al. (1997) Sulfated galactocerebrosides as potential antiinflammatory agents. J. Med. Chem. **40:** 3234–3247

90 Laskowitz D., Goel S., Bennett E. R. and Matthew W. D. (1997) Apolipoprotein E suppresses glial cell secretion of TNFα. J. Neuroimmunol. **76:** 70–74

91 Finch C. E. and Sapolsky R. M. (1999) The evolution of Alzheimer's disease, the reproductive schedule and apoE isoforms. Neurobiol. Aging **20:** 407–428

92 Dallongeville J., Lussier-Cacan S. and Davignon J. (1992) Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. J. Lipid Res. **33:** 447–454

93 Utermann G. (1987) Apolipoprotein E polymorphism in health and disease. Am. Heart J. **113:** 433–440

94 Hallman D. M., Boerwinkle E., Saha N., Sandholzer C., Menzel H. J., Csazar A. et al. (1991) The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am. J. Hum. Genet. **49:** 338–349

95 Roheim P. S., Carey M., Forte T. and Vega G. L. (1979) Apolipoproteins in human cerebrospinal fluid. Proc. Natl. Acad. Sci. USA **76:** 4646–4649

96 Montine T. J., Montine K. S. and Swift L. L. (1997) Central nervous system lipoproteins in Alzheimer's disease. Am. J. Pathol. **151:** 1571–1575

97 Montine K. S., Bassett C. N., Ou J. J., Markesbery W. R., Swift L. L. and Montine T. J. (1998) Apolipoprotein E allelic influence on human cerebrospinal fluid apolipoproteins. J. Lipid Res. **39:** 2443–2451

98 Rebeck G. W., Alonzo N. C., Berezovska O., Harr S. D., Knowles R. B., Growdon J. H. et al. (1998) Structure and functions of human cerebrospinal fluid lipoproteins from individuals of different APOE genotypes. Exp. Neurol. **149:** 175–182

99 Guyton J. R., Miller S. E., Martin M.E., Khan W. A., Roses A. D. and Strittmatter W. J. (1998) Novel large apolipoprotein E-containing lipoproteins of density 1.006-1.060 g/ml in human cerebrospinal fluid. J. Neurochem. **70:** 1235–1240

100 Mulder M., Ravid R., Swaab D. F., de Kloet E. R., Haasdijk E. D., Julk J. et al. (1998) Reduced levels of cholesterol, phospholipids and fatty acids in cerebrospinal fluid of Alzheimer disease patients are not related to apolipoprotein E4. Alz. Dis. Assoc. Disord. **12:** 198–203

101 Yamauchi K., Tozuka M., Hidaka H., Hidaka E., Kondo Y. and Katsuyama T. (1999) Characterization of apolipoprotein E-containing lipoproteins in cerebrospinal fluid: effect of phenotype on the distribution of apolipoprotein E. Clin. Chem. **45:** 1431–1438

102 Yamauchi K., Tozuka M., Nakabayashi T., Sugano M., Hidaka H., Kondo Y. et al. (1999) Apolipoprotein E in cerebrospinal fluid: relation to phenotype and plasma apolipoprotein E concentrations. Clin. Chem. **45:** 497–504

103 Fagan A. M., Younkin L. H., Morris J. C., Fryer J. D., Cole T. G., Younkin S. G. et al. (2000) Differences in the Abeta40/Abeta42 ratio associated with cerebrospinal fluid lipoproteins as a function of apolipoprotein E genotype. Ann. Neurol. **48:** 201–210

104 Eckert G. P., Kirsch C. and Mueller W. E. (2001) Differential effects of lovastatin treatment on brain cholesterol levels in normal and ApoE-deficient mice. Neuroreport **12:** 883–887

105 Hayashi H., Igbavboa U., Hamanaka H., Kobayashi M., Fujita S. C., Wood W. G. et al. (2002) Cholesterol is increased in the exofacial leaflet of synaptic plasma membranes of human apolipoprotein E4 knock-in mice. Neuroreport **13:** 383–386

106 Kirsch C., Eckert G. P., Koudinov A. R. and Mueller W. E. (2003) Brain cholesterol, statins and Alzheimer's Disease. Pharmacopsychiatry **36** (Suppl. 2): S113–S119
107 Howland D. S., Trusko S. P., Savage M. J., Reaume A. G., Lang D. M., Hirsch J. D. et al. (1998) Modulation of secreted beta-amyloid precursor protein and amyloid beta-peptide in brain by cholesterol. J. Biol. Chem. **273:** 16576–16582
108 Refolo L. M., Pappolla M. A., Malester B., LaFrancois J., Bryant-Thomas T., Wang R. et al. (2000) Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. Neurobiol. Dis. **7:** 321–331
109 Levin-Allerhand J. A., Lominska C. E. and Smith J. D. (2002) Increased amyloid-levels in APPSWE transgenic mice treated chronically with a physiological high-fat high-cholesterol diet. J. Nutr. Health Aging **6:** 315–319
110 Tietz A., Oron L., Mazliah E. and Michaelson D. M. (2002) The effects of apolipoprotein E genotype on brain lipid metabolism. Adv. Behav. Biol. **51:** 33–38
111 Mauch D. H., Naegler K., Schumacher S., Goertzz C., Mueller E.-C., Otto A. et al. (2001) CNS synaptogenesis promoted by glia-derived cholesterol. Science **294:** 1354–1357
112 Pfrieger F. W. (2003) Cholesterol homeostasis and function in neurons of the central nervous system. Cell. Mol. Life Sci. **60:** 1158–1171
113 Dietschy J. M. and Turley S. D. (2001) Cholesterol metabolism in the brain. Curr. Opin. Lipidol. **12:** 105–112
114 Michikawa M., Fan Q.-W., Isobe I. and Yanagisawa K. (2000) Apolipoprotein E exhibits isoform-specific promotion of lipid efflux from astrocytes and neurons in culture. J. Neurochem. **74:** 1008–1016
115 Gong J.-S., Kobayashi M., Hayashi H., Zou K., Sawamura N., Fujita S. C. et al. (2002) Apolipoprotein E (apoE) isoform-dependent lipid release from astrocytes prepared from human apoE3 and apoE4 knock-in mice. J. Biol. Chem. **277:** 29919–29926
116 Montine T. J, Montine K. S., Olson S. J., Graham D. G., Roberts L. J. II., Morrow J. D. et al. (1999). Increased cerebral cortical lipid peroxidation and abnormal phospholipids in aged homozygous apoE-deficient C57BL/6J mice. Exp. Neurol. **158:** 234–241
117 Igbavboa U., Hamilton J., Kim H.-Y., Sun G. Y. and Wood W. G. (2002) A new role for apolipoprotein E: modulating transport of polyunsaturated phospholipid molecular species in synaptic plasma membranes. J. Neurochem. **80:** 255–261
118 Han X., Holtzman D. M. and McKeel D. W. Jr (2001) Plasmalogen deficiency in early Alzheimer's disease subjects and in animal models: molecular characterization using electrospray ionization mass spectrometry. J. Neurochem. **77:** 1168–1180
119 Poirier J., Hess M., May P. C. and Finch C. E. (1991) Astrocytic apolipoprotein E mRNA and GFAP mRNA in hippocampus after entorhinal cortex lesioning. Brain Res. Mol. Brain Res. **11:** 97–106
120 Stone D. J., Rozovsky I., Morgan T. E., Anderson C. P., Hajian H. and Finch C. E. (1997) Astrocytes and microglia respond to estrogen with increased apoE mRNA in vivo and in vitro. Exp. Neurol. **143:** 313–318
121 Stoll G., Meuller H. W., Trapp B. D. and Griffin J. W. (1989) Oligodendrocytes but not astrocytes express apolipoprotein E after injury of rat optic nerve. Glia **2:** 170–176
122 Nakai M., Kawamata T., Taniguchi T., Maeda K. and Tanaka C. (1996) Expression of apolipoprotein E mRNA in rat microglia. Neurosci. Lett. **211:** 41–44
123 Xu P. T., Gilbert J. R., Qiu H. L., Rothrock-Christian T., Settles D. L., Roses A. D. et al. (1998) Regionally specific neuronal expression of human APOE gene in transgenic mice. Neurosci. Lett. **246:** 65–68