

Lipid homeostasis and apolipoprotein E in the development and progression of Alzheimer's disease

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Abstract Extracellular amyloid plaques, intracellular neurofibrillary tangles, and loss of basal forebrain cholinergic neurons in the brains of Alzheimer's disease (AD) patients may be the end result of abnormalities in lipid metabolism and peroxidation that may be caused, or exacerbated, by β -amyloid peptide ($A\beta$). Apolipoprotein E (apoE) is a major apolipoprotein in the brain, mediating the transport and clearance of lipids and $A\beta$. ApoE-dependent dendritic and synaptic regeneration may be less efficient with apoE4, and this may result in, or unmask, age-related neurodegenerative changes. The increased risk of AD associated with apoE4 may be modulated by diet, vascular risk factors, and genetic polymorphisms that affect the function of other transporter proteins and enzymes involved in brain lipid homeostasis. Diet and apoE lipoproteins influence membrane lipid raft composition and the properties of enzymes, transporter proteins, and receptors mediating $A\beta$ production and degradation, tau phosphorylation, glutamate and glucose uptake, and neuronal signal transduction. The level and isoform of apoE may influence whether $A\beta$ is likely to be metabolized or deposited. This review examines the current evidence for diet, lipid homeostasis, and apoE in the pathogenesis of AD. Effects on the cholinergic system and response to cholinesterase inhibitors by APOE allele carrier status are discussed briefly.—Lane, R. M., and M. R. Farlow. Lipid homeostasis and apolipoprotein E in the development and progression of Alzheimer's disease. *J. Lipid Res.* 2005. 46: 949–968.

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Genetic disorders of amyloid production and metabolism may produce early-onset forms of Alzheimer's disease (AD). However, the most common form of the disease is sporadic and late onset, with an as yet unknown cause. Dietary factors may be important in the pathogenesis of AD (1–3), with PUFAs and MUFAs and n-3 double bonds [in ω -3 essential fatty acids (EFAs)] conferring protection and an excess of saturated fats or n-6 double bonds (in ω -6 EFAs) increasing the risk (4). Dietary intake of EFAs is re-

flected in the lipid composition of the brain, which is critical for maintaining membrane integrity, electrical insulation, vesicular trafficking, and synaptic neurotransmission. In addition, it has been proposed that the inhibition of lipid metabolism by high-carbohydrate diets may be the most detrimental aspect of modern diets (5). AD may be similar to obesity, coronary artery disease (CAD), and type II diabetes mellitus in being a consequence of the conflict between our Paleolithic genetic constitution and our current Neolithic diet.

Diet may interact with apolipoprotein E (apoE) isoforms, such as apoE4, which may also suppress lipid metabolism, to determine the risk and rate of sustained lipid autoperoxidation within cellular membranes and the effectiveness of membrane repair. Diet, insulin signaling, membrane lipid composition, apoE genotype and levels, amyloid processing and trafficking, β -amyloid peptide ($A\beta$) oligomer damage to lipid membranes, oxidative stress, and intracellular neurofibrillary tangle (NFT) formation are interrelated by many mechanisms (5–7). For example, apoE expression and isoform influence the composition of membrane lipid rafts, which affects the structure and function of membrane proteins involved in glucose and neurotransmitter transport, the trafficking of $A\beta$, and the phosphorylation of tau and its polymerization into intracellular NFTs (8–10). Alterations in apoE-modulated trafficking of $A\beta$ may have dramatic effects on whether the peptide is metabolized or begins to deposit within the brain. ApoE4 is associated with increased peripheral lipid levels, decreased cerebral glucose metabolism, increased glial activation and excitotoxicity resulting in greater inflamma-

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; apoE, apolipoprotein E; APP, amyloid precursor protein; $A\beta$, β -amyloid peptide; BBB, blood-brain barrier; BuChE, butyrylcholinesterase; CAD, coronary artery disease; ChE-I, cholinesterase inhibitor; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; EFA, essential fatty acid; HMG-CoA-R, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; LDLR, low density lipoprotein receptor; LRP, low density lipoprotein receptor-related protein; NFT, neurofibrillary tangle; NMDA, N-methyl-D-aspartate; TG, triglyceride.

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tion and oxidative stress, less effective sequestration of heavy metals, and increased A β deposition and NFT formation in the brain before the onset of symptoms of dementia. This review examines the putative roles and interrelationships between apoE, lipid homeostasis, brain glucose metabolism, and the trafficking of A β in the pathogenesis and progression of AD and other dementias. Dietary changes, perhaps particularly in individuals with an APOE ϵ 4 allele, may significantly delay or effectively prevent AD, and therapeutic interventions that restore lipid homeostasis may treat the disease.

DIET AND LIPID HOMEOSTASIS

The hypothesis presented in this section is that diets high in carbohydrate, particularly those with a high glycaemic index, and low in EFAs, particularly ω -3 long-chain PUFAs, increase the risk for developing AD. Although these diets may also increase the risk of vascular disease, the AD risk may be mediated through effects on lipid metabolism and neuronal membrane lipid composition that induce changes in glucose transport, neurotransmission,

antioxidant defenses, inflammatory responses, cerebral blood flow, and cognitive functioning. These effects may be modulated by an apoE isoform, such as apoE4, which, like a high-carbohydrate diet, decreases lipoprotein lipase activity and inhibits the delivery of FFA to glia and to neurons (Fig. 1).

Fatty acids

Whereas cholesterol in the brain is produced and regulated endogenously, the fatty acid content of the brain is strongly influenced by diet (11). The phospholipid composition of neuronal membranes, such as the ratio of membrane ω -3 to ω -6 long-chain PUFAs, can be modulated by dietary intake (12), apoE expression (13), and the presence of AD pathology (14, 15) (Fig. 1). EFAs, the precursors of these long-chain PUFAs, cannot be synthesized by animals and must be obtained through the diet. The phospholipids of nerve endings and synaptic vesicles are rich in long-chain PUFAs, such as the ω -3 long-chain PUFA docosahexaenoic acid (DHA), whose precise composition determines the fluidity and flexibility of membranes and modulates the structure and function of membrane-associated proteins (16). Particularly in the presence of genetic risk for

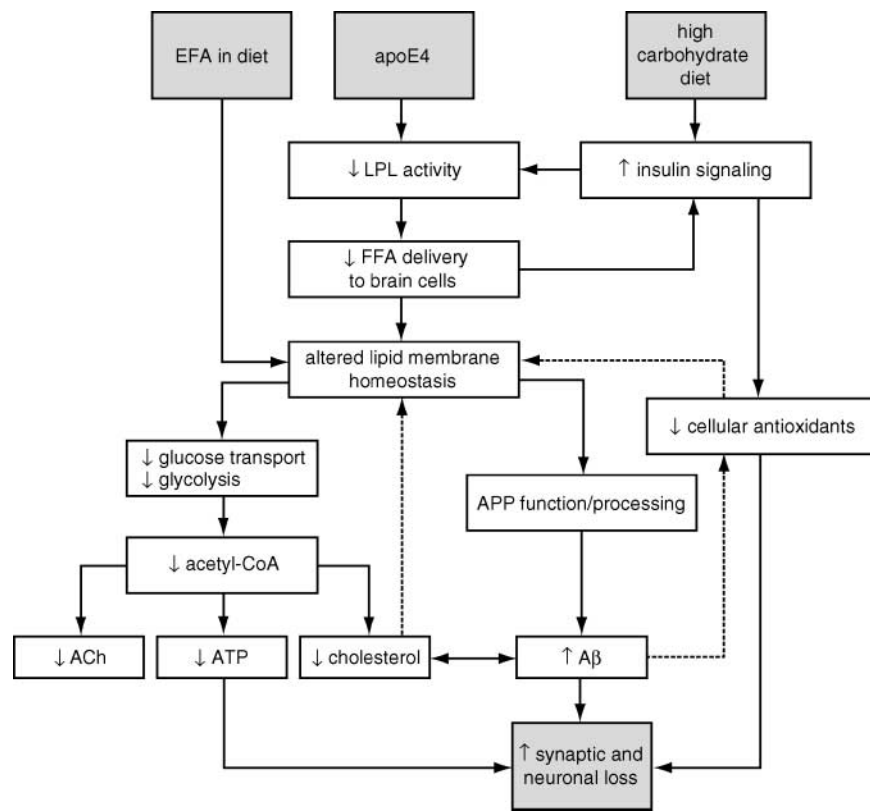


Fig. 1. Inhibition of lipid metabolism by apolipoprotein E (apoE) gene (APOE) ϵ 4 and a high-carbohydrate diet inhibits the delivery of FFA to brain cells. This, along with deficient or imbalanced dietary essential fatty acid (EFA) content, may alter lipid membrane homeostasis, which inhibits the function of neuronal glucose transporters and alters the function and processing of amyloid precursor protein (APP). Decreased glycolysis lowers acetyl-CoA-derived ATP, acetylcholine (ACh), and cholesterol levels. Altered lipid membrane homeostasis affects APP processing and increases the production of β -amyloid peptide (A β). Chronic excessive insulin signaling further decreases LPL activity and increases cellular damage by, for example, decreasing cellular antioxidative stress responses. Ultimately, these disruptions result in the increasing cellular dysfunction, synaptic loss, and neuronal loss characteristic of Alzheimer's disease (AD).

AD-type pathology, diets deficient in ω -3 may be associated with decreased synaptic membrane DHA levels, membrane lipid peroxidation, loss of postsynaptic proteins, synaptic loss and production of neuronal apoptotic products (15, 17), and inefficient function of membrane proteins such as glucose transporters (18). Decreased cerebral glucose utilization is one of the earliest signs of AD and may be evident in at-risk populations, such as APOE ϵ 4 carriers, well before clinical signs of dementia occur (19). ApoE4 is associated with the suppression of lipid metabolism, inducing inefficient delivery of EFAs such as DHA to cerebral neurons, resulting in inhibited function of glucose transporters (5) (Fig. 1).

Changes in the ratios of ω -6 to ω -3 long-chain PUFAs in modern diets, related to increased consumption of vegetable oils and meats (which are rich in ω -6 long-chain PUFAs), are considered by some to be a cause of the increased incidence of CAD and psychiatric disorders, such as depression (20). ω -6 EFAs are metabolized via arachidonic acid to proinflammatory eicosanoids, compared with anti-inflammatory eicosanoids generated from the ω -3 family via DHA and eicosapentaenoic acid (21–23). The relative proportions of membrane DHA/eicosapentaenoic acid and arachidonic acid depend on the relative availability of their respective precursors, α -linoleic acid and linoleic acid, as both ω -3 and ω -6 EFAs compete for the same enzyme pathways. Thus, diets high in α -linoleic acid are anti-inflammatory, whereas diets high in linoleic acid are proinflammatory. Dietary intake of ω -3 long-chain PUFAs and consumption of fish, which is the primary source of DHA (the most abundant component of membrane phospholipid in metabolically active areas of the brain, such as cerebral cortex, mitochondria, synaptosomes, and synaptic vesicles), may also reduce the risk of incident AD (3, 15, 24, 25).

These long-chain PUFAs are precursors of a large number of highly active eicosanoids and both are important components of the postreceptor signal transduction systems for most neurotransmitters, cytokines, and growth factor receptors. Given the importance of fatty acid/eicosanoid signal transduction in all excitable tissues, it would not be surprising if disturbances in this system were to lead to neurological problems. The balance of dietary ω -6/ ω -3 long-chain PUFAs may influence levels of neurotransmitters, such as glutamate, acetylcholine (ACh), and dopamine (26–30), levels of nerve growth factor (31), synaptic membrane function (32, 33), the function of membrane proteins (13, 18), oxidative stress, lipid peroxidation, antioxidative defense (15, 17, 34–36), glutamate-induced excitotoxicity (37), cerebral blood flow (38, 39), ischemic damage (40), blood pressure (41), and cognitive functions (27, 33, 42–45).

Carbohydrate

A well-defined risk factor for late-onset AD is possession of one or more alleles of the ϵ 4 variant of the apoE gene (APOE). APOE ϵ 4 allele frequencies are low in populations with long historical exposure to agriculture (46), suggesting that consumption of a high-carbohydrate diet may have selected against APOE ϵ 4 carriers. Populations

with the lowest frequencies of APOE ϵ 4 include long-time agriculturalists, such as Greeks (6.8%), Turks (7.9%), Mayans (8.9%), and Arabs living in northern Israel (4%), whereas populations with the highest frequencies include long-time hunter-gatherers, such as African Pygmies (40.7%), Papuans (36.8%), and Inuits (21.4%) (47, 48). The APOE ϵ 4-encoded protein (apoE4) and a high-carbohydrate diet may both suppress lipid metabolism in a similar manner (5). APOE ϵ 4 by itself increases the risk for early-onset CAD (48), and in combination with a high-carbohydrate diet, the risk of CAD, and perhaps also of AD, is greatly increased.

High-carbohydrate diets increase insulin levels, increase insulin and insulin growth factor signaling, decrease lipoprotein lipase activities, inhibit the uptake of free fatty acids by cells, and increase the serum “residence” time of triglyceride (TG)-rich lipoproteins, such as chylomicrons and VLDLs (49, 50). The rate of clearance of TG-rich lipoproteins and the uptake of FFAs depend mainly on the activity of lipoprotein lipases and are strongly influenced by insulin signaling (50). The hydrolysis of core TGs by lipoprotein lipase in chylomicrons and VLDLs to produce cholesterol-rich remnant particles is necessary to achieve their apoE-mediated clearance from plasma. Similar to a high-carbohydrate diet, the apoE4 protein increases TG-rich lipoprotein residence time by inhibiting lipolysis (51). It does this by binding to TG-rich lipoprotein more avidly than apoE2 or apoE3, displacing apoC-II, which is required for maximal rates of lipoprotein lipase catalysis, and resulting in decreased lipoprotein lipase activity (52, 53). In the brain, decreased lipoprotein lipase activity inhibits the delivery of FFA to glia and to neurons (Fig. 1). Inefficient delivery of EFAs to neurons leads to inhibited function of glucose transporters. The hippocampus is especially vulnerable to glucose insufficiency (54).

As apoE4 and high-carbohydrate diets both inhibit lipid metabolism, this may explain the selection against the APOE ϵ 4 allele in populations with a long historical exposure to agriculture. CAD is likely to have been the selective force, because it generally occurs earlier than AD. Increased glucose and insulin levels resulting from high carbohydrate consumption induce lipogenesis and hypertriglyceridemia. Calorie restriction is more effective at reducing hyperlipidemia in type 2 diabetic patients with the APOE ϵ 4 allele (55). The APOE ϵ 4 allele may not be inherently damaging but only in combination with a high-carbohydrate diet, which is damaging in itself and is likely to be a major contributor to the high risk of CAD, and possibly AD, in modern populations with or without the APOE ϵ 4 allele.

In summary, diets deficient in ω -3 EFAs, or that contain excess ω -6/ ω -3 ratios, appear to increase the risk of developing AD. Dietary intake of EFAs is reflected in the fatty acid composition of neuronal membranes in the brain. This composition modulates the structure and function of membrane-associated proteins, such as the glucose transporter. Decreased cerebral glucose utilization is one of the earliest signs of AD. A high-carbohydrate diet, similar to apoE4, suppresses lipid metabolism and reduces the deliv-

ery of EFAs to cells in the brain. Disturbances in lipid metabolism within the brain compromise the integrity of cell membranes, decreasing the function of membrane proteins. Mild chronic increases of insulin/insulin-like growth factor signaling accelerate damage to cortical neurons. Potential consequences of a high-carbohydrate diet include both AD and insulin resistance, which is also associated with cognitive impairment and functional decline (56).

Cholesterol

Brain cell synthesis, efflux, and influx of cholesterol are tightly regulated. Neuronal synaptic requirements for cholesterol needed in membrane lipid raft composition, plasticity, and regeneration are met through the uptake of apoE-cholesterol complexes produced by glia in addition to axonal transport of cholesterol produced in cell bodies. A β production, tau hyperphosphorylation, and lipid peroxidation may represent acute physiological reactions to impaired cholesterol homeostasis, chronic disturbance of which may result in AD. Although brain cholesterol homeostasis appears to be isolated from the rest of the body by the blood-brain barrier (BBB), high serum cholesterol levels may increase brain iron levels and vulnerability to oxidative stress. In addition, products of peripheral cholesterol catabolism can cross the BBB and increase apoE expression. Interventions (e.g., possibly statins) that may restore cholesterol homeostasis, improve lipid metabolism, and/or normalize apoE levels could prevent or treat AD (57).

In the brain. Approximately 25% of the total amount of cholesterol in the human body is found in the brain, mostly in the specialized membranes of myelin and in the membranes of neuronal and glial cells. Almost all brain cholesterol is a product of local synthesis, with the BBB efficiently protecting it from exchange with lipoprotein cholesterol in the circulation (58). Thus, there is a highly efficient apoE-dependent recycling of cholesterol in the brain, including a potentially large turnover between neurons and glial cells (59), with minimal losses to the circulation. Under steady-state conditions, de novo synthesis of cholesterol in the brain is largely balanced by excretion of 24S-hydroxycholesterol, which is capable of escaping the brain recycling mechanism and crossing the BBB (60). Serum and cerebrospinal fluid (CSF) 24S-hydroxycholesterol are increased in early AD, possibly as a result of increased brain cholesterol turnover during neurodegeneration (61). The severity of AD is independently associated with reductions in the serum 24S-hydroxycholesterol-cholesterol ratio, possibly reflecting a loss in the protective effect of neuronal cholesterol 24S-hydroxylase-mediated catabolism of cholesterol in the central nervous system (62).

Cholesterol homeostasis in brain cells is balanced between cholesterol influx after internalization of apoE-rich lipoprotein complexes bound to the cell surface and intracellular cholesterol synthesis via the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA-R) pathway. More than 20% of hippocampal neuronal polysynaptic clusters turn over within 24 h (63), and cholesterol plays an essential role in synaptic plasticity. However, unlike other

membrane lipids, cholesterol cannot be synthesized at neuronal terminals. Thus, it is likely that synaptic function depends on whether cholesterol is fully supplied from endogenous sources (i.e., transport via axonal flow) (64) or from exogenous sources (i.e., uptake, via lipoprotein receptors, of apoE-cholesterol complexes produced by astroglia) (65). Cholesterol levels and cholesterol turnover are affected in neurodegenerative disorders, and the capacity for cholesterol transport and recycling in the brain seems to be important for the development of such diseases.

Inefficient delivery of EFAs to cerebral neurons decreases the function of glucose transporters. This results in a decreased pool of acetyl-CoA, as the cell derives this almost exclusively from glucose. Neuronal acetyl-CoA pools are used in the synthesis of ACh and also of cholesterol. A reduction may result in decreased levels of ACh and disturbed cholesterol homeostasis, a consequence of which is the improper processing of amyloid precursor protein (APP) and the generation of A β (66). APP may function as a membrane cargo receptor during axonal transport, delivering several cellular factors, including β -secretase, presenilin 1, and neurotrophin receptors (67–69). Whether as a result of a mutation in APP, the enzymes that process APP, or disturbed cholesterol homeostasis, the premature cleavage of APP may disrupt cellular trafficking (70), the functional regulation of the glial glutamate transporters (71), the delivery neurotrophin receptors to the cell surface (72), and copper homeostasis, resulting in oxidative stress (73).

Disturbances in cholesterol homeostasis in either direction may result in AD pathology. Lack of cholesterol supply to neurons impairs neurotransmission and synaptic plasticity (65), inducing neurodegeneration and tau pathology (74, 75). High free intracellular cholesterol results in HMG-CoA-R-mediated biosynthesis of cholesterol being suppressed, but this is also accompanied by the reduction of several basic cellular processes, chronic inhibition of which results in cytoskeletal changes, alterations in tau phosphorylation, NFT formation, and eventually apoptosis (76). On the basis of this mechanism, a statin that entered the brain would not be expected to have a beneficial role in preventing the onset of AD. Generation and clearance of A β are regulated by cholesterol (77), with free cholesterol decreasing and cholesteryl esters, which are formed by the esterification of cytoplasmic cholesterol droplets, increasing cellular A β production. Increased intracellular cholesteryl ester concentrations lead to the inhibition of (nonamyloidogenic) α -secretase activity but stimulate (amyloidogenic) β - and γ -secretase cleavage of APP. Conversely, oligomeric A β can reduce the cholesterol level in neurons by promoting cholesterol release from the neurons to generate apoE lipoprotein-A β complexes (78) and may also inhibit cholesterol synthesis (79). Loss of neuronal membrane cholesterol may also contribute to excessive amyloidogenesis in AD (10).

Oligodendrocytes are responsible for synthesizing a significant proportion of the cholesterol in the brain. In so doing, oligodendrocytes contribute not only to the forma-

tion of myelin sheaths but also to plasticity and learning, which depend upon the elaboration of axonal and dendritic processes that contain significant amounts of cholesterol. Cholesterol biosynthesis requires iron as a cofactor in key synthetic steps, such as HMG-CoA-R. HMG-CoA-R is enriched in oligodendrocytes relative to other cells (80). Oligodendrocytes are very sensitive to a variety of toxic insults, including oligomeric A β -induced lipid peroxidation, traumatic brain injury, hypertension, anoxia, and hypercholesterolemia (81). Oligodendrocytes have a very high basal metabolism, as they must produce large amounts of lipid to construct the myelin sheath. This, of course, sets the stage for oxidative stress and cell death within these unique glial elements. The glutamate-mediated excitotoxic death of oligodendrocytes occurs predominantly by necrosis, releasing large amounts of iron, and contributes to pathogenesis in demyelinating disease (82, 83). There is growing appreciation of the process of demyelination of brain white matter in the development and progression of AD.

Oligodendrocyte pathology may play a primary role in AD pathology, and a model of oligodendrocyte degeneration in AD involving relationships between cholesterol synthesis, A β monomers and oligomers, apoE, iron, reactive oxygen species, oligodendrocyte differentiation, and microglial activation is evolving (84, 85). Oligodendrocytes produce A β (86), and as myelination progresses, the increasing numbers of oligodendrocytes can directly increase A β levels and can indirectly increase them through the provision of more cholesterol. Oligodendrocyte cholesterol production may drive the age-related increase in whole brain cholesterol levels that peak in the fourth decade of life (87). The increasing availability of cholesterol means that the cholesterol content of the exofacial leaflet of brain cell membranes doubles with age (88). This may induce an age-related increase in toxicity, as the production and oligomerization of A β is promoted by the high cholesterol content of lipid bilayers (89). However, the increasing A β oligomer-membrane interactions progressively remove membrane cholesterol, and markedly reduced brain cholesterol levels are eventually seen in individuals who progress to AD (90).

In the periphery. The effect of peripheral cholesterol concentrations, such as high serum LDL or low serum HDL, on brain cholesterol is controversial. Brain cholesterol content does appear to be affected, perhaps as a result of the stability of cholesterol in myelin, but it has not been established whether intramembranous lipid domains or intracellular cholesterol content are affected. Increased serum cholesterol levels are also associated with increases in markers of brain neuronal oxidative stress, including antioxidant defenses and by-products of lipid peroxidation, and microglial and astrocyte activation (91–93). Oxidative stress may target lipid rafts that contain, among other membrane proteins, APP and γ -secretase. Lipid rafts play an important role in A β biogenesis by regulating the β -secretase pathway (94). Increased generation and secretion of A β leads to the formation of oligomeric and aggregated A β . Chronic increases in serum total cholesterol also increase apoE mRNA levels in brain and in-

crease glial cell and secreted apoE levels (95). Studies in animal models have shown that diet-induced hypercholesterolemia increases A β and apoE concentrations in temporal and frontal cortices, but not in cerebellum, and that these regional increases parallel the amyloid pathology observed in the AD brain (96). ApoE expression by astrocytes may be regulated by oxysterols, which are hydroxylation products of cholesterol that include 24S and 25S-hydroxycholesterol. Oxysterols found in the circulation are able to pass the BBB and may activate apoE expression through nuclear receptor signaling (97). This mechanism provides a potential explanation for how serum cholesterol levels may influence brain cholesterol homeostasis.

Some of the effect of the APOE ϵ 4 allele on the risk of AD may be mediated through high serum cholesterol (98). ApoE contributes more to normal cholesterol variability than any other gene identified to date in cholesterol metabolism (99). In general, apoE2 decreases total cholesterol levels and apoE4 increases them. Increased serum cholesterol could result in increased endocytic transfer of serum transferrin-bound iron into endothelial cells and therefore increased transport of iron across the BBB (100). In the brain, iron is concentrated predominantly in glial cells, and increased iron staining in oligodendrocytes is seen in the brains of rabbits fed high-cholesterol diets (100). This increase in iron may further increase the vulnerability of the brain to oxidative stress. High serum cholesterol levels in midlife may be a risk factor for early amyloidogenesis and the development of AD (101, 102). Thus, serum cholesterol may exert its effect at an early stage of cognitive decline, and the association may no longer be apparent by the time cognitive function has deteriorated sufficiently to be classified as dementia (101). Although increased cholesterol in the brain, perhaps attributable to peripheral hypercholesterolemia, may be the initiating event, it may not be required, and aging and other factors, including apoE4, may be sufficient.

Recent retrospective epidemiological studies have reported that the use of HMG-CoA-R inhibitors (statins), but not nonstatin lipid-lowering agents, may reduce the risk of developing AD (103, 104). Some recent prospective studies have not yielded similarly encouraging results (105), but issues of adequate dosage, sufficient follow-up, and inherent bias (in that only patients with cholesterol levels warranting treatment received statins) mean that the place of statins in the prevention of AD remains unknown. A recent prospective observational study suggested that statin use may not decrease the risk of AD in the overall population but may do so in those younger than 80 years with an APOE ϵ 4 allele (106). Alternatively, it has been suggested that statins may slow the progression of AD (107), perhaps as a result of neuroprotective properties rather than any inhibition of A β production (108). Statins decrease serum LDL and 24S-hydroxycholesterol and increase serum HDL (60). Although APOE ϵ 2 carriers show greater reductions in LDL cholesterol in response to statins and APOE ϵ 4 carriers are the least responsive (109, 110), APOE ϵ 4 carriers have a greater reduction in adverse cardiovascular outcomes (111). Statins also decrease

circulating TG-rich lipoprotein by increasing receptor-mediated uptake and increasing levels of lipoprotein lipase (112). Thus, statins may directly counteract the effects of high-carbohydrate diets and insulin resistance on lipid metabolism. Different statins, regardless of their brain availability, induce alterations in cellular cholesterol distribution in the brain (113). Pleiotropic statin-induced effects include upregulation of brain endothelial nitric oxide (111), effects on iron transport (100), reduced inflammation (114, 115), decreased glial cell apoE secretion affecting apoE-mediated A β deposition, and upregulation of membrane apoE receptors (95). These may be cholesterol synthesis-independent effects that are indirectly mediated at the BBB or via statin-induced reductions of levels of intermediates on the way to cholesterol, such as isoprenoids. The latter has been suggested as responsible for the increased nonamyloidogenic processing of APP by α -secretase (116), reduced A β -induced microglial inflammatory responses, including A β -induced interleukin 1 β and inducible nitric oxide synthase expression, reduced nitric oxide production (114), and reduced glial cell apoE secretion (115).

In summary, all mammalian cells require cholesterol for the formation and maintenance of cell membrane permeability and fluidity. Most extrahepatic cells are unable to catabolize cholesterol and therefore need to export it to maintain sterol homeostasis. Cellular cholesterol levels are precisely controlled by biosynthesis, efflux from cells, and influx of lipoprotein cholesterol into cells. The BBB efficiently prevents the exchange of brain cholesterol with peripheral cholesterol. Cholesterol is synthesized in the brain but not at nerve terminals. It plays an essential role in synaptic plasticity and must be fully supplied from axonal transport or via uptake of apoE-cholesterol complexes produced by glia. Inefficient EFA and glucose delivery to neurons can result in disturbed cholesterol homeostasis. This may result in improper processing of the membrane protein APP, A β generation, oxidative stress, and altered cell trafficking. Indeed, changes in amyloid processing, the "pathogenicity" of A β , neuronal cytoskeleton changes, tau formation, and oxidative stress reactions may repre-

sent acute physiological mechanisms to compensate for impaired cholesterol homeostasis and/or associated neurotransmission and synaptic plasticity failure. Chronic disturbances in lipid homeostasis may result in AD. Improved delivery of EFAs to neurons, reduced brain iron load, and decreases in glial secreted apoE levels are among the many putative mechanisms whereby statins might reduce the risk of AD.

There is growing appreciation of the process of demyelination of brain white matter in the development and progression of AD. Oligodendrocytes are a uniquely vulnerable cell population in the brain to a variety of toxic insults because of the very high basal metabolism required to produce large amounts of cholesterol to construct the myelin sheath. Oligodendrocyte degeneration in AD may involve relationships between cholesterol synthesis and homeostasis, A β monomers and oligomers, apoE, iron, glutamate-mediated excitotoxicity, and reactive oxygen species. The onset of AD may be prevented or delayed if the structural and functional integrity of oligodendrocytes, and therefore of myelin, is maintained into old age. A model for the role of altered membrane lipid metabolism in the pathogenesis of AD is shown in Fig. 2.

LIPID PEROXIDATION

High oxygen consumption, a low capacity for regeneration, increasing metal ion concentrations and declining antioxidant defenses with age, and the vulnerability of long-chain PUFA-containing membranes to lipid peroxidation result in brain tissue being highly susceptible to free radical-induced injury. Lipid peroxidation leads to altered membrane characteristics, with generation of A β that induces more oxidative stress and calcium influx that induces glutamate excitotoxicity and cell death.

Oxidative damage results when oxidative stress (i.e., oxidation of lipids, protein, and DNA) exceeds the antioxidant capacity of tissue. Oxidative stress is the result of imbalances in pro-oxidant/antioxidant homeostasis that

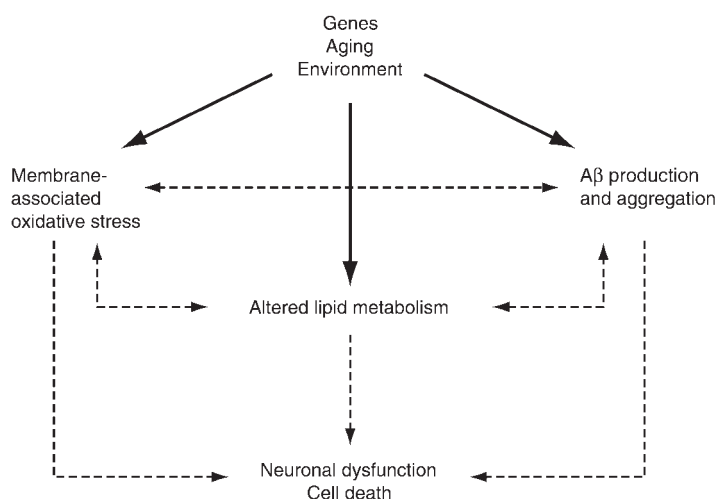


Fig. 2. Model for the role of altered membrane lipid metabolism in the pathogenesis of AD. The aging process, in combination with genetic and environmental/dietary factors, results in the following: 1) increased production and accumulation of neurotoxic forms of A β , which itself alters lipid metabolism, exacerbates oxidative stress, and is neurotoxic; 2) increases in cellular cholesterol attributable to altered synthesis, efflux, or influx trigger synaptic dysfunction, increase oxidative stress, further A β production and aggregation, and may induce neuronal death; and 3) increasing metal ion concentrations, poor antioxidant defenses, and altered PUFA content of membranes increase vulnerability to free radical-induced injury. Altered membrane characteristics caused by lipid peroxidation generate more A β and calcium influx, inducing glutamate excitotoxicity and cell death. ApoE may protect cells against oxidative stress by delivering cholesterol and EFAs to cells, clearing A β , reducing glial cell activation, limiting glutamate excitotoxicity, and sequestering heavy metal ions.

lead to the generation of toxic reactive oxygen species. High oxygen consumption, relatively low antioxidant levels, and low regenerative capacity result in brain tissue being more susceptible to oxidative damage. DNA damage may reduce the expression of selectively vulnerable genes involved in learning, memory, and neuronal survival, initiating a program of brain aging that starts early in adult life (117). Mounting evidence implicates regionally increased oxidative damage to the brain beyond what occurs with aging as a key process contributing to disease progression in AD (118). The brain is rich in long-chain PUFAs that are particularly vulnerable to oxidative modification, and lipid peroxidation is a sensitive marker of oxidative stress. Low antioxidant (119) and low DHA (15, 24) intake are two identified risk factors for oxidative neurodegeneration that may synergize.

Lipid membrane oxidation may be the essential first step in a cascade of parallel interacting processes that involve the expression of A β and NFTs (4). Putatively, disturbances in the homeostasis of cholesterol, itself a source of oxidative stress (92), may initiate amyloid formation, which in its oligomeric form is a potent source of oxidative stress. An initial free radical-induced injury may induce a vicious cycle in which amyloidogenic processing of APP would be further enhanced, generating more A β , which in turn would cause more oxidative stress. Thus, once membrane lipids have been exposed to free radicals, a process of autoperoxidation may be initiated that may, if uninterrupted, proceed for years (120). Structural damage to membranes and generation of oxidized products are the two main outcomes of lipid peroxidation. Oxidative stress differentially affects brain regions and the cholinergic system, as levels of peroxidizable unsaturated lipids, antioxidant defenses, and levels of membrane-bound proteins differ between brain regions.

Over time, the autoperoxidation process changes the lipid composition of the membrane, as long-chain PUFAs are lost faster than they can be replaced. The loss of long-chain PUFAs leads to a reduction in membrane fluidity and altered function of membrane-embedded proteins, receptors, and calcium channels (121, 122). These changes affect membranes, including those of cells, vascular endothelia, and those around cellular organelles. Oxidized lipid and free radicals in the cytoplasm induce failure in the lipid membranes of organelles such as mitochondria and lysosomes. The myriad consequences of this damage include decreased energy metabolism and lysosomal enzyme leakage into the neuronal cytoplasm. The production of soluble A β may be a defense against oxidative stress, but oligomeric forms of A β may induce further free radical oxidative stress, including protein oxidation, lipid peroxidation, and DNA oxidation (123). A β and hyperphosphorylated tau contribute to an accelerating cycle of neuronal damage that induces further inflammatory responses and oxidative degeneration (124). The final step in the cascade is neuronal cell death.

The generation of free radicals requires the activation of molecular oxygen. Organisms have evolved a range of metalloenzymes to take advantage of the interaction be-

tween oxygen and the multiple valence states of metal ions, such as iron, copper, and zinc, to generate reactive oxygen species. Free radicals are an intrinsic part of normal metabolism. However, they are also toxic, and cells have developed many elaborate means of regulating both the metal ion interactions and the generation of free radicals. Although the normal cell harnesses the production of free radicals for beneficial means, the process becomes destructive when the regulatory system breaks down. Proteins implicated in several age-dependent neurodegenerative diseases such as A β and α -synuclein might abnormally present Cu²⁺ or Fe³⁺ ligands for inappropriate reaction with oxygen (73). One of the consequences of normal aging is an increase in metal ions in the brain. This may increase the likelihood that proteins such as A β will harness endogenous biometals to foster inappropriate free radical generation. The importance of iron overload as a catalyst for oxidative stress with lipids in the risk of developing AD was recently demonstrated in a population-based study (125). When both cholesterol and transferrin saturation were increased, the risk of AD was much greater than when either of these factors was increased alone.

A β and oxidative products, such as reactive aldehydes, inhibit the uptake of glutamate into neurons and glia by the glutamate transporter and the breakdown of glutamate (126–128). This inhibition may be reversed by antioxidants (127). A downstream effect of free radical-induced calcium influx is a glutamate receptor-mediated excitotoxic response (129) that triggers a cascade of events leading to cell death. Excitotoxic responses are a feature of a number of neurological conditions, including ischemia, epilepsy, AD, Parkinson's disease, and amyotrophic lateral sclerosis (130). ApoE directly protects neurons and glia against irreversible oxidative injury by reducing glial activation and limiting secondary glutamate excitotoxicity and the secretion of inflammatory mediators (131–135). ApoE also sequesters trace metals such as iron, which promote oxidative stress (136), but apoE4 may be less able to bind and detoxify heavy metals (57).

The human brain has a particularly high degree of metabolic activity and concomitant energy needs associated with maintaining a heavily myelinated brain. High metal ion concentrations maintain many of its functions, but a poor capacity to cope with oxidative stress and little regenerative capacity make the brain very susceptible. Diets deficient in antioxidants and DHA, and aging or aging with disease, may affect or be affected by this process. Key proteins in neurodegenerative disease, such as A β and α -synuclein, may be involved in aberrant reduction/oxidation metal interactions with the induction of oxidative stress. Oxidative stress manifests a variety of toxicities, including a vicious cycle of structural damage to membranes, inflammation and oxidative degeneration, glutamate-mediated excitotoxicity, and cell death. ApoE may have a role in protecting cells from oxidative stress, which is discussed in greater detail below, through the clearance of A β , the sequestration of metals, and the regulation of glial activation. The effectiveness of apoE isoforms may differ in this regard.

The APOE $\epsilon 4$ allele, in a “gene-dose” effect, increases the risk and decreases the age of onset of AD. ApoE4 is associated with increased peripheral lipid levels, reduced peripheral lipid metabolism, decreased cerebral glucose metabolism, greater glial activation and excitotoxicity, resulting in more inflammation and oxidative stress, less sequestration of heavy metals, decreased A β clearance, increased A β aggregation and deposition, and NFT formation in the brain before the onset of symptoms of dementia. ApoE is a major apolipoprotein in the brain, mediating the transport, delivery, and clearance of cholesterol and phospholipids. These processes regulate the cholesterol and PUFA content of synaptic membranes and are dependent on the expression and isoform of apoE. The formation and plasticity of synaptic connections requires that neurons import glia-derived cholesterol via apoE- and cholesterol-containing lipoproteins. ApoE-dependent regeneration and remodeling of dendritic and synaptic circuitry after neuronal injury may be less efficient in carriers of the APOE $\epsilon 4$ allele, and this may result in, or lead to, earlier unmasking of age-related cholinergic neuronal loss. The response to cholinesterase inhibitor (ChE-I) treatment may be modified by APOE $\epsilon 4$ allelic status. The increased risk of AD associated with the APOE $\epsilon 4$ allele may be modulated by diet, vascular risk factors, and the function of other transporter proteins and enzymes involved in brain lipid homeostasis. The list of genes related to lipid transport and catabolism that have polymorphisms linked to AD continues to grow. ApoE lipoproteins influence membrane lipid raft composition and properties of enzymes, transporter proteins, and receptors mediating A β production and degradation, tau phosphorylation, glutamate and glucose uptake, and neuronal signal transduction. Thus, lipid rafts may be an important site where A β , apoE, and tau interact in a way that influences the formation of A β fibrils and paired helical fragments of phosphorylated tau. ApoE also mediates the transport, fibrilization, and clearance of A β , and the level and isoform of APOE may influence whether A β is likely to be metabolized or deposited.

In peripheral and brain lipid homeostasis

Lipoproteins are lipid transport vesicles that ensure the solubility of lipids within aqueous biological environments. Lipoproteins are composed of a phospholipid and a free cholesterol shell surrounding a TG and cholesteryl ester core. Apolipoproteins stabilize the surface of lipoproteins, serve as cofactors for enzymatic reactions, and serve as ligands for lipoprotein receptors. The soluble apolipoprotein gene family, which includes apoE, encodes proteins with amphipathic structures that allow them to exist at the water-lipid interface (137). In humans, apoE is a 299 amino acid, 35 kDa glycoprotein that has three major isoforms that differ at two residues: E2, E3, and E4 at frequencies of 7–8%, 77–78%, and 14–15%, respectively, in the general population (138). These single amino acid changes result in functional differences between the apoE

isoforms, including their relative affinities for both apoE receptors and lipoprotein subtypes (139) and their stability and tendency to form reactive folding intermediates (140). ApoE4 binds preferentially to large, TG-rich VLDLs, whereas apoE2 and apoE3 bind preferentially to small, phospholipid-rich HDLs.

ApoE plays a major role in the transport of lipids in the bloodstream, where it participates in the delivery and clearance of serum TGs, phospholipids, and cholesterol. ApoE-containing lipoproteins are bound and internalized via receptor-mediated endocytosis by a number of proteins in the low density lipoprotein receptor (LDLR) and LDLR-related protein (LRP) families. These receptors may exhibit different affinities for the three common apoE isoforms (141). For example, lipoproteins associated with apoE4, such as VLDLs and TG-rich remnant lipoproteins, are removed faster from plasma than those containing apoE3 and apoE2. Cholesterol input from these lipoproteins induces a downregulation of LDLR and thus a higher concentration of circulating cholesterol (141). It has also been suggested that apoE4-associated hyperlipidemia may be a consequence of deficient lipoprotein lipase activity and/or reduced affinity/visibility of lipoprotein-bound apoE4 (142). The apoE2 and apoE4 phenotypes are associated with increased levels of plasma TGs relative to apoE3. ApoE2 results in decreased plasma LDL, whereas increased LDL concentrations are associated with apoE4. ApoE concentrations are generally higher in hypertriglyceridemia than in hypercholesterolemia (143). Concentrations of apoE are usually lower in APOE $\epsilon 4$ carriers (143).

ApoE is expressed within the brain predominantly by astrocytes, oligodendrocytes, and microglia (144); it has an essential role in the normal secretion of glial lipoproteins and is isolated from the circulation by the BBB. Neurons express receptors for apoE. Thus, apoE may be involved in the delivery and clearance of cholesterol and phospholipids to, for example, neurons and myelin-synthesizing oligodendrocytes. In response to nerve injury, cholesterol synthesis in neurons is inhibited (145) and there is increased neuronal expression of apoE (146). At the same time, glial cell apoE synthesis and lipid transport from glial cells to neurons are increased, so that cholesterol is available to repair the injured neurons (147). Steady-state intracellular levels of apoE reflect a balance between the synthesis and degradation of both newly synthesized and recycled apoE, whereas secreted apoE levels reflect a balance between apoE released from the glia and reuptake of apoE via apoE receptors that are expressed on the cell surface. ApoE4 may be more susceptible to pathogenic degradation within neurons, reducing the increase of this isoform in response to injury (148). Moreover, the usually protective response of increased apoE expression may be detrimental in APOE $\epsilon 4$ carriers as a result of the increased formation of C-terminal-truncated apoE fragments that stimulate tau phosphorylation and the formation of intracellular NFTs (149).

ApoE is involved in the regulation of cholesterol in the outer (exofacial) and inner (cytofacial) leaflets, and in the PUFA content of phospholipid molecular species of the

synaptic membrane (13). Changes in synaptic membrane lipid composition result in altered function of membrane-bound enzymes. ApoE lipoproteins may also participate in the efflux of cholesterol from the neuronal cell surface, particularly under circumstances of cellular degeneration. ABCA1, a member of the ATP cassette binding superfamily, is a transmembrane cholesterol and phospholipid transporter that is widely expressed throughout the body. Outside the central nervous system, ABCA1 mediates the efflux of cholesterol and phospholipids to HDLs. Deficiency of ABCA1 results in a lack of circulating HDLs. In the brain, ABCA1 is required for cholesterol efflux to apolipoprotein, including apoE (150). In both astrocytes and microglia, ABCA1 deficiency decreases lipid efflux to exogenous apoE, increases glial lipid accumulation, reduces the lipidation of apoE, and decreases apoE levels in whole brain and particularly in the striatum and hippocampus (150). The decreased apoE levels in the central nervous system are not related to APOE gene expression but likely result from the increased metabolism of abnormally lipidated apoE-containing lipoprotein (151).

In dementia

Genetic epidemiological studies have shown that the APOE $\epsilon 4$ allele confers an increased risk and decreases the age of onset of AD in a gene-dose-dependent manner, whereas APOE $\epsilon 2$ might play a protective role (152). However, others have suggested that APOE polymorphism does not simply modify the risk of Alzheimer-like changes in brain but, more specifically, regulates the pace of their progression. Thus, although APOE $\epsilon 3$ and $\epsilon 2$ do not alter the proportion of the population that is susceptible to AD, they may slow the progression of neurodegeneration in vulnerable individuals (153). The APOE $\epsilon 4$ allele is associated with a more rapid rate of cognitive decline before the clinically symptomatic phase of dementia begins (154). Less effective regeneration and remodeling by apoE4 may result in an earlier unmasking of age-associated synaptic and neuronal loss. There is an apparent interaction between the APOE genotype and sex (155, 156). For example, the age-specific risk of AD in older women with the vulnerable $\epsilon 4/\epsilon 4$ genotype is more than double that of men. Women who are heterozygous for $\epsilon 4$ are also at increased risk relative to men, but the presence of some apoE3 or apoE2 protein attenuates the sex effect considerably. Only in women with no inherited apoE4 is the risk of AD equal to that in men.

Patients with probable AD, late-middle-aged $\epsilon 4$ carriers, and young (20–39 years) healthy adult $\epsilon 4$ carriers all have abnormally low rates of cerebral glucose metabolism bilaterally (19). At early stages, deficits may be masked by recruiting larger regions of the brain to accomplish tasks (157). The APOE $\epsilon 4$ allele may be associated with cognitive impairment in AD through an association with NFTs and a greater A β burden in the form of diffuse and neuritic plaques (158). The effect of APOE $\epsilon 4$ on AD incidence or cognitive decline has been reported to increase significantly in patients with concurrent cerebrovascular disease (159), hypertension, diabetes, hypercholesterol-

emia, or atherosclerosis (103, 160–162), and with genetic defects in intracellular lipid trafficking from endocytosed lipid particles, and in membrane and brain cholesterol removal (7, 163, 164). Interestingly, in AD, there is a loss of the elevating effect of apoE4 on serum cholesterol, TGs, and phospholipids (165). Of the various direct and indirect mechanisms suggested for the pathophysiological actions of apoE4, it is not known to what extent each contributes to the pathogenesis of AD.

In the AD brain, the de novo synthesis of cholesterol and lipid molecules is reduced in cortical and hippocampal areas, and neurons are dependent on the internalization of exogenous apoE-rich lipoprotein complexes that bind to cell surfaces. ApoE may also mediate the removal of lipid through cell membrane efflux, and if this lipid is oxidized, the removal and delivery of oxidized lipid within lipoprotein complexes may result in the accumulation of lipid peroxidation products in neurons and glia (166). ApoE may have a role in protecting brain membranes from A β -induced oxidative stress, with apoE2 and apoE3 being more efficient than apoE4. ApoE deficiency is associated with chronic oxidative stress, enhanced lipid peroxidation, and alterations in membrane lipids (167–169). This suggests that inefficient delivery of fatty acids to the membrane may underlie the susceptibility to oxidative stress. It has been suggested that both increased cytokine concentration and decreased cholesterol concentration in the AD brain could reduce apoE levels, and in doing so accelerate neurodegeneration (170). However, higher levels of apoE have been observed in the brains and plasma of AD patients compared with age-matched controls (98, 171, 172). In addition, genetic epidemiological studies have observed associations between polymorphisms in the APOE gene promoter that confer greater transcriptional activity and an increased risk of late-onset AD (reviewed in 173). Thus, although apoE is quickly upregulated in response to various types of neuronal injury, suggesting a neuroprotective role, overexpression of apoE may be neurotoxic (174). Although excess apoE4 may be undesirable, it has been suggested that the expression of apoE3 provides a dose-dependent protective effect against A β deposition (175). However, it has also been suggested that an increased expression of apoE may generally be protective, regardless of isoform (176).

A β is produced by normal cells and usually exists as a soluble monomeric molecule and has diverse functions in the brain (177). A β is amphipathic and, although it has a high tendency to self-associate, to oligomerize, and to form fibrils, under normal conditions A β prefers to associate with lipoproteins (178). It has been suggested that A β is a normal structural-functional apolipoprotein constituent of apoE-containing lipoproteins or HDLs in both the brain and the circulation (179). ApoE plays an important role in regulating the metabolic pathway that controls the elimination of soluble A β in the brain extracellular space (180). There appears to be a very dynamic equilibrium between CSF and plasma A β that is almost certainly modulated by apoE and modified by A β deposition (175). Altered trafficking of A β might have a dramatic effect on whether the

peptide is metabolized or begins to deposit within the brain. A β not associated with lipoproteins may induce neurotoxicity or glial activation or be deposited in neuritic plaques. The deposition of A β in plaques may occur when its transport and clearance by apoE lipoprotein fails. In the periphery, A β can be metabolized in association with TG-rich lipoprotein (181), and in the brain, cellular clearance is via apoE receptor-mediated endocytosis of apoE/A β complexes by either neurons or glia (182–184).

Given the same amount of A β production, the density of neuritic plaques appears to be dependent on apoE level (185, 186). This may be attributable to the isoform-dependent role of apoE as a pathological chaperone for A β , which involves apoE not only in its clearance but also in its fibrilization. It has been suggested that apoE4 specifically enhances the nucleation and aggregation of A β deposits and that the processes of disaggregation or conversion to fibrillar deposits are stimulated similarly by the different apoE isoforms (187). Lipidated apoE4 appears less effective in removing extracellular A β , and lipid-free apoE4 is more effective at promoting A β fibril formation and zinc- and copper-induced A β aggregation (182, 188, 189). Thus, the presence of apoE4, rather than other apoE isoforms, may result in increased A β -mediated oxidative damage to synaptosomes, A β deposition, fibrilization, and neuritic plaque formation (190). Moreover, A β induces glial cell activation and increased secretion of apoE. LRP mediates A β -induced glial activation, whereas LDLR mediates the A β -induced changes in apoE levels. Astrocyte-specific expression of apoE markedly affects A β deposition, and alterations in the ability of astrocytes to degrade A β , attributable to either aging or APOE genotype, or both, could be involved in the pathogenesis of AD (184).

The A β -stimulated increases of glial apoE appear to limit the inflammatory response of glia to A β (191). Although A β stimulation of glial apoE may limit neuroinflammation, overproduction of apoE by activated glia might exacerbate inflammation with more robust proinflammatory activity with apoE4 rather than apoE3. This provides yet another mechanistic link between the APOE ϵ 4 allele and AD (191). Greater astrogliosis in the presence of apoE4 may be a consequence of increased soluble and insoluble A β aggregates (187). Significantly greater gliosis, A β deposition, levels of CSF excitatory amino acids, energy metabolism, and slower recovery with greater functional disability are seen in patients with an APOE ϵ 4 allele after injury and ischemia and also in patients with neurodegeneration (192–194). After an ischemic event, infarct size is larger and functional disability is greater in the presence of an APOE ϵ 4 allele (195). Patients with apoE4 may be more vulnerable to developing white matter lesions in the presence of vascular risk factors such as hypertension (196). Thus, factors that mediate glial activation, proliferation, and maturation, including butyrylcholinesterase (BuChE), acetylcholinesterase (AChE), peroxisome proliferator-activated receptor activation, high peripheral cholesterol levels, injury, ischemia, EFAs and their derivatives, and A β , may interact with apoE isoforms to mediate glia-induced inflammation and oxidative stress in the brain

(93, 191, 194, 197–199). Chronic gliosis has been shown to trigger altered amyloid processing in vivo (200), and the pathological changes are associated with AD (201).

In modulating response to ChE-Is

Cholinergic function is now well known to be impaired in patients with AD, and the loss of cholinergic neurons, particularly in the limbic system and cortex, is a hallmark of the disease. A β _{1–42} demonstrates exceptionally high-affinity binding to the nicotinic α 7 ACh receptors that are expressed most abundantly on the cell surface of cholinergic and cholinceptive neurons (e.g., cortical pyramidal cells), providing a plausible explanation for the selective vulnerability of these cells in AD (202). Diet may also influence the synthesis of ACh (Fig. 1), and decreased hippocampal and cortical, but not cerebellar, AChE levels have been demonstrated in animal models, particularly females, receiving a high-carbohydrate or a high-fat diet (203). ApoE ϵ 4 carriers with AD show greater deficits than noncarriers in cholinergic activity in the hippocampus and cortex, a greater reduction in the total number of cholinergic neurons, and loss of cholinergic markers such as choline acetyltransferase activity and nicotinic ACh receptor binding (204–206). Greenwood et al. (207) speculated that reduced efficiency of the cholinergic projections from basal forebrain to parietal and temporal cortices could underlie the impairments of visual attention seen in their study of healthy, middle-aged carriers of the APOE ϵ 4 gene.

It has also been suggested that patients with the APOE ϵ 4 allele may show greater rates of disease progression (208). However, imaging studies have found that APOE ϵ 4 may accelerate the progression of hippocampal atrophy in prodromal and in early AD, but once an individual is advanced in age or in the progression of the disease, any influence of APOE ϵ 4 on the rate of progression is lost (209). In fact, it has been shown that the rate of cerebral atrophy in established AD subjects with a mean age of 70 years may be slower in association with APOE ϵ 4 relative to other genotypes (210), whereas in older patients, with a mean age of 80 years (209), progression was no different by genotype (211). This is supported by clinical data that demonstrate that during the prodromal phase of AD, carriers of an APOE ϵ 4 allele may progress faster than noncarriers (154, 212). In mild disease, progression may be comparable or faster, and in more advanced stages, progression may be slower in APOE ϵ 4 carriers relative to noncarriers (Fig. 3) (unpublished data on file, ADENA database, Novartis Pharmaceuticals). In patients with mild AD, noncarriers of APOE ϵ 4 show no cognitive decline on placebo over a 6 month period and appear less responsive to ChE-I treatment. This is in contrast to the decline on placebo and the significant treatment effects seen in APOE ϵ 4 carriers. These differences may be related to the greater cholinergic deficits in APOE ϵ 4 carriers with mild disease. Non-APOE ϵ 4 carriers may not manifest significant cholinergic deficits until later in the disease.

ChE-I treatment effects may differ with respect to clinical and biomarker outcomes in AD patients with and without an APOE ϵ 4 allele (213). These anti-dementia agents

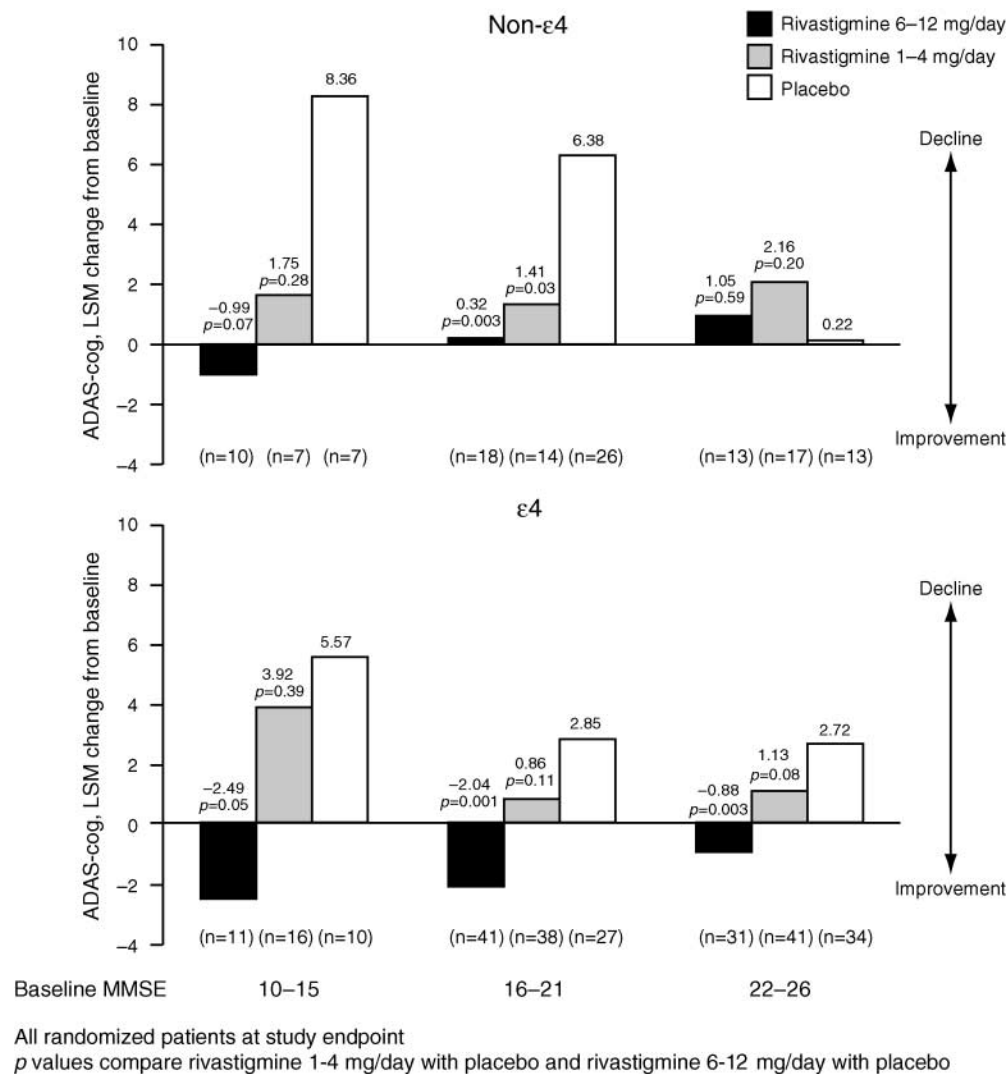


Fig. 3. Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog) least square means (LSM) change from baseline after rivastigmine 6-12 mg/day, rivastigmine 1-4 mg/day, or placebo for 6 months in patients with mild to moderate AD (who consented to genotype determination in the pivotal registration studies for rivastigmine) by baseline disease severity and APOE $\epsilon 4$ status (all randomized patients) (unpublished data on file, ADENA database, Novartis Pharmaceuticals).

differ with respect to their ability to inhibit BuChE in addition to AChE and whether the inhibition of target enzymes is sustained (214). Rivastigmine and galantamine appeared to achieve similar quantitative responses in both carriers and noncarriers of APOE $\epsilon 4$ in retrospective analyses of placebo-controlled trials in patients with mild to moderate AD (215, 216). However, the response of patients with two APOE $\epsilon 4$ alleles may vary among ChE-Is (217, 218). **Figure 4** shows the responses to recommended doses of rivastigmine versus low doses of rivastigmine and placebo in mild to moderate AD patients with a mean age of 74.1 years by APOE $\epsilon 4$ carrier status (215; unpublished data on file, ADENA database, Novartis Pharmaceuticals). Patients with two APOE $\epsilon 4$ alleles had a mean age of 72.5 years and appeared to show a greater response than heterozygotes and noncarriers of the $\epsilon 4$ allele. The reason for this differential responsiveness is unclear, because sim-

ilar AD pathology might be anticipated regardless of genotype, and the finding requires confirmation.

AChE, BuChE, and APOE all have roles in glial activation (191, 197, 198) and neurite outgrowth (146, 218). Both AChE and APOE have roles in A β fibrilization (188, 219), and BuChE and APOE have roles in lipid metabolism and transport (13, 220-222). The BuChE-K polymorphism may also influence the risk of AD conferred by the APOE $\epsilon 4$ allele (223, 224) and may be associated with slower disease progression in patients with moderate AD (225, 226). In patients with mild AD treated with rivastigmine or tacrine for 1 year, the rivastigmine group showed no change in tau concentrations in the CSF, in contrast to significant increases in both tacrine-treated and untreated AD subjects (213). These CSF tau changes were seen mainly in APOE $\epsilon 4$ carriers. Interestingly, more extensive white matter lesions are exhibited by APOE $\epsilon 4$ homozygotes

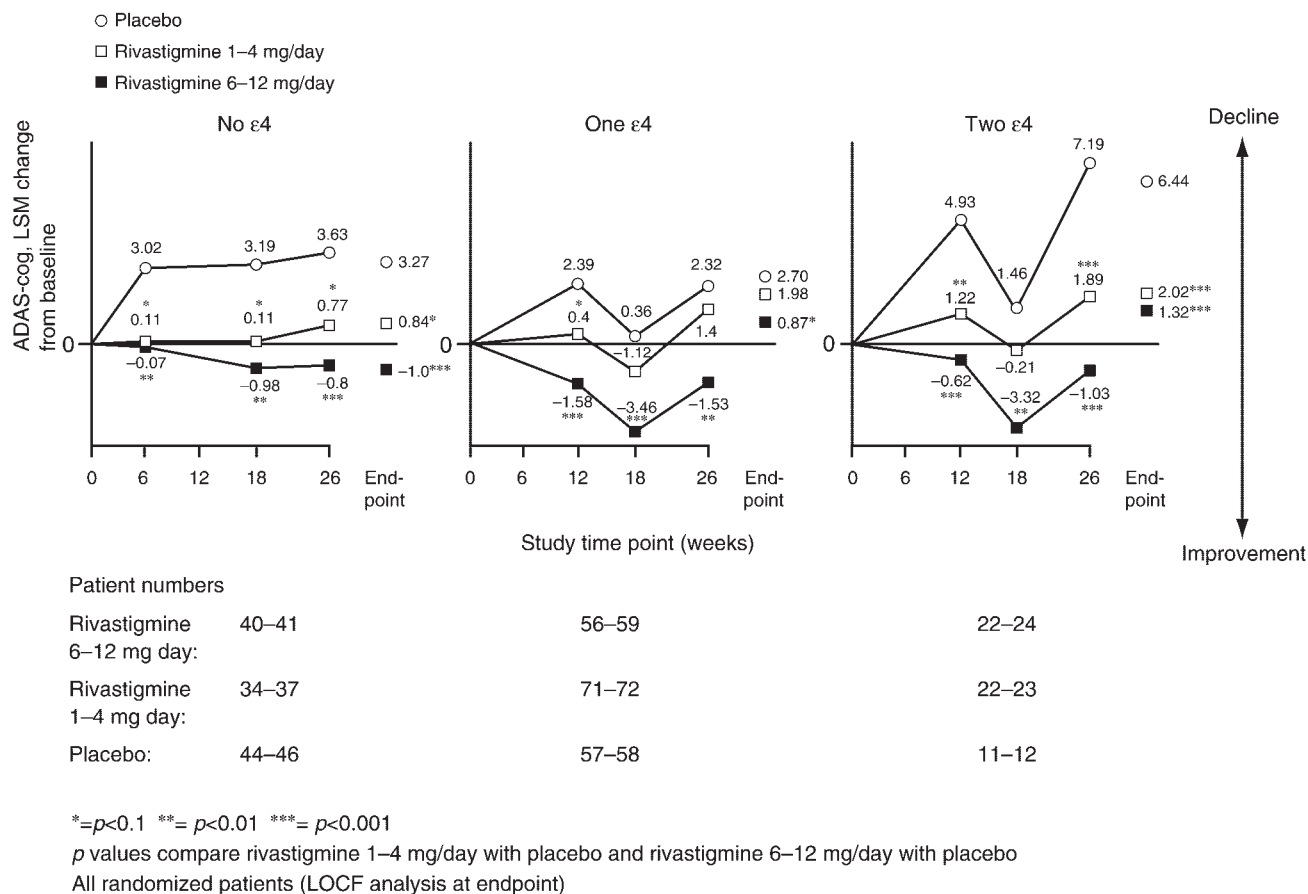


Fig. 4. Least square means (LSM) change in Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog) 11 item scores over 6 months in mild to moderate AD patients receiving rivastigmine 1–4 mg/day (open squares), rivastigmine 6–12 mg/day (closed squares), or placebo (open circles) by apoE allelic status [all randomized patients; last observation carried forward (LOCF) analysis at end point] (unpublished data on file, ADENA database, Novartis Pharmaceuticals).

than by other genotypes (227). It has been suggested that rivastigmine may be effective in treating subcortical ischemic vascular dementia (228–231). Thus, in patients with two APOE ϵ 4 alleles, ChE-Is may treat both the cholinergic deficit associated with AD pathology and the subcortical ischemic pathology that may be more likely in these patients.

In lipid rafts

Lipid rafts are dynamic assemblies of cholesterol and sphingolipids on the outer leaflet of the membrane that play an important role in signal transduction and other cellular functions (232). Sphingolipids are the main component of membrane lipid rafts. The synthesis of sphingolipids requires fatty acids and the nonessential amino acid L-serine. Glutamate and glycine, as a result of increased neuronal activity, may increase L-serine release from astrocytes (233). This L-serine enhances the release of glial apoE (234). The cholesterol- and fatty acid-containing lipoproteins and the L-serine are taken up by neurons. L-Serine and fatty acids are used to increase the synthesis of sphingolipids and phosphatidylserine and, along with the cholesterol, promote synaptogenesis and neuronal survival (65). Glia-derived apoE may directly influence neuronal cell signaling through binding to neuronal cell surface

apoE receptors (235), or astrocytes may exert control over neuronal function indirectly through the effects of glia-derived cholesterol and phospholipids on lipid raft composition and the function of membrane proteins, such as the glutamate transporter (236).

APP, γ -secretase, β -secretase, neprilysin, GM-1 ganglioside, and cholesterol are all found in lipid rafts and may be associated in a functionally meaningful way (i.e., it is likely that lipid rafts play an important role in A β production and degradation). Small diffusible A β oligomers might form in lipid rafts, where they would be well positioned to cause toxicity by interfering with signal transduction. Lipid rafts may be an important site where A β , apoE, and tau interact in a way that influences A β fibril formation and the formation of paired helical fragments of phosphorylated tau (237). For example, A β fibrils do not form, and lipid raft A β is decreased, when apoE is absent (238, 239), and fibril formation is accelerated by the presence of apoE4 compared with other apoE forms (240). A β oligomers can inhibit hippocampal long-term potentiation, and in animal models the age-dependent accumulation of dimeric A β , accompanied by apoE and eventually by phosphorylated tau, occurs at a time when memory impairment begins (237).

Furthermore, it has also been suggested that apoE receptors functionally interact with APP to control cellular signaling and tau phosphorylation (for review, see 241). ApoE4 has altered affinity for apoE receptors and may interfere with survival-promoting functions that these receptors might have in neurons. A simple, plausible mechanism by which this could occur would be competition for the binding of signaling proteins that interact with neurons through apoE receptors. Furthermore, apoE receptors interact with other important transmembrane protein receptors, such as the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor, through their cytoplasmic domains. The NMDA receptor is crucial to the regulation of synaptic development, the long-term potentiation of synaptic strength, and plasticity in the central nervous system.

In summary, apoE is involved in the transport, delivery, and clearance of cholesterol and phospholipids in the periphery and in the brain. Affinity for, and potential to downregulate, LDLR is isoform-dependent and may account for the different serum lipid profiles across apoE phenotypes. In addition, apoE4 protein also increases TG-rich lipoprotein serum residence time by inhibiting lipolysis through a reduction in lipoprotein lipase activity. In the brain, apoE is involved in the delivery and clearance of cholesterol and phospholipids to, for example, neurons and myelin-synthesizing oligodendrocytes. ABCA1 is required to maintain normal levels of apoE in the brain. ApoE regulates neuronal membrane cholesterol and the PUFA content of synaptic membrane phospholipids. ApoE isoforms may differ in their ability to deliver fatty acids and cholesterol to neurons and in their ability to mediate lipid efflux. In addition to genotype, apoE levels determine how it influences both normal and pathological processes. The formation and plasticity of synaptic connections require that neurons import glia-derived cholesterol via apoE- and cholesterol-containing lipoproteins. This apoE-dependent regeneration and remodeling of dendritic and synaptic circuitry after neural injury or neurodegeneration is less efficient in carriers of the APOE ϵ 4 allele, and this may result in early unmasking of the neuronal loss associated with aging.

The APOE ϵ 4 allele, in a gene-dose effect, increases the risk and decreases the age of onset of AD. ApoE4 is associated with increased peripheral lipid levels and reduced lipid metabolism as well as with decreased glucose metabolism and increased A β deposition and NFT formation in the brain before the onset of symptoms of dementia. Furthermore, apoE4 is associated with greater fibrilization and less efficient apoE lipoprotein-mediated clearance of A β . Neurons and glia use LRP-mediated internalization to scavenge extracellular A β -lipoprotein complexes and, once internalized, send them to the endosomal and lysosomal compartments for degradation. The lower expression of apoE4 may result in reduced clearance of A β . Furthermore, the presence of apoE4 relative to other isoforms may result in greater inflammation and oxidative stress as a result of greater glial activation and excitotoxicity. ApoE4 may also be more susceptible to pathogenic degradation within neurons, with increased formation of neuro-

toxic fragments that stimulate NFT formation. In addition to a greater propensity to develop AD pathology, subcortical pathology may be more likely to develop in APOE ϵ 4 carriers after head injury and in the presence of vascular risk factors such as hypertension. Responsiveness to ChE-Is by APOE ϵ 4 carrier status requires further investigation.

Lipid rafts are dynamic assemblies of cholesterol and sphingolipids on the outer leaflet of the membrane. Increased neuronal activity causes astrocytes to release L-serine, which induces glia to secrete apoE lipoprotein. The cholesterol and fatty acids from this lipoprotein influence lipid raft composition and the properties of enzymes, transporter proteins, and receptors that mediate A β production and degradation, tau phosphorylation, glutamate uptake, and neuronal signal transduction. Thus, lipid rafts may be an important site where A β , apoE, and tau interact in a way that influences A β fibril formation and the formation of paired helical fragments of phosphorylated tau. In addition, the apoE receptors (all LDLR family members) are pivotal mediators of signaling events that maintain synaptic plasticity and neuronal survival. ApoE binding may interfere with these other important functions that are routed through the same receptors.

DISCUSSION

Dietary factors may interact with disease-causing or predisposing genes in molecular cascades that either promote or prevent abnormalities in lipid metabolism and lipid peroxidation attributable to, or exacerbated by, A β (5, 130). Evolutionarily discordant diets may eventually result in or accelerate the development of AD. Disturbances in lipid metabolism within the central nervous system inhibit the functions of membrane proteins such as glucose transporters and APP. Prolonged excessive insulin/insulin-like growth factor signaling accelerates cellular damage in cerebral neurons. These factors suggest several preventative and treatment strategies. A change in diet emphasizing decreasing dietary carbohydrates and increasing EFAs, such as DHA, may effectively prevent or delay AD. Interventions that restore lipid homeostasis may treat the disease, including drugs that increase fatty acid metabolism, EFA repletion therapy, and ketone body treatment. The protective effects of diet may be greater in patients carrying the APOE ϵ 4 allele. Luchsinger et al. (242) reported that hazard ratios of AD in ϵ 4 carriers with high calorie and fat intake were significantly greater than those of individuals with low calorie and fat intake, whereas hazard ratios for patients not carrying the ϵ 4 allele were not significantly different. Obesity modulates the association between the APOE genotype and fasting insulin and glucose levels, particularly in men (243). Although weight control is important in all people, it may be especially important in men with an APOE ϵ 4 allele to modify potentially increased fasting insulin and glucose levels. Dietary recommendations partly based on genetic factors may help to reduce AD risk more efficiently than universal recommendations.

Carrying the APOE ϵ 4 allele is not sufficient for the development of AD, and individuals with one or two ϵ 4 alleles may reach late old age without cognitive impairment. High serum cholesterol levels, or diets high in carbohydrate and/or low in ω -3 or ω -3/ ω -6 EFA ratio, may modify the risk associated with APOE ϵ 4 carrier status, as may other sources of disrupted brain lipid homeostasis and vascular risk factors such as hypertension. The APOE ϵ 4 allele acts as a potent risk factor for AD by accelerating onset. However, the risk of AD appears heterogeneous in ways independent of APOE. Some individuals seem destined to escape AD, even over an extended lifespan (244). Only approximately half of APOE ϵ 4 homozygotes develop AD by the age of 90 years (245). It is estimated that the lifetime risk of developing AD increases to 29% for carriers of one ϵ 4 allele and is 9% for those with no ϵ 4 allele (246). In the brain, apoE-containing lipoproteins are involved in lipid delivery, for example to neurons undergoing synaptic remodeling or replacement and dendrite outgrowth, and in lipid clearance. As basal forebrain cholinergic neurons are lost with age, the cholinergic innervation of the cortex can be maintained by increasing the dendritic tree and synaptic connections of the remaining cholinergic neurons. However, apoE4 may be less efficient at this remodeling, which may result in earlier failure of cortical cholinergic innervation. Delivered cholesterol and fatty acids also influence lipid raft composition and the properties of enzymes, transporter proteins, and receptors that mediate A β production and degradation, tau phosphorylation, the uptake of glutamate and glucose, and neuronal signal transduction. Thus, lipid rafts may be an important site where A β , apoE, and tau interact.

Amyloid plaques and NFTs may be the products of causative processes implicating oxidation and inflammation and involving abnormalities of lipid metabolism and dietary lipid intake. For example, the ratio of ω -3 to ω -6 dietary EFAs significantly affects the balance of proinflammatory and anti-inflammatory eicosanoids, and the ratio of saturated to unsaturated fatty acids determines the fluidity, functionality, and oxidative susceptibility of lipid membranes. Similar to a high-carbohydrate diet, apoE4 increases the serum residence time of TG-rich lipoprotein by decreasing lipoprotein lipase activity. In the brain, decreased lipoprotein lipase activity inhibits the delivery of FFA to glia and to neurons, and this may result in inefficient glucose transport and increased oxidative stress. The hippocampus is especially vulnerable to glucose insufficiency. Furthermore, by failing to protect transported lipid from oxidation via altered clearance, aggregation, and deposition and toxicity of A β , one or more APOE ϵ 4 alleles may markedly increase the risk of developing AD. ApoE may be required for the clearance of soluble extracellular A β but may also (particularly apoE4) mediate the nucleation and aggregation of A β by acting as a pathological chaperone. The absolute level of expression and the particular isoform of the apoE molecule may have the potential to influence both A β fibrillogenesis and metabolism to differing extents.

Interventions involving dietary carbohydrate and lipids,

and in lipid metabolism, show great promise in slowing or possibly averting the development of AD, including dietary changes, cholesterol- and TG-modifying agents, and antioxidants. Opportunities for further research include epidemiological studies of the interaction between dietary carbohydrate, EFAs, and other fats and the APOE ϵ 4 allele. Aggressive intervention studies of combinations of dietary modification, antioxidants, and cholesterol- and/or TG-lowering agents may be warranted in individuals at high risk of developing AD. The relationship between serum and brain lipid homeostasis, diet, vascular risk factors, and response to ChE-I treatment in patients with AD also requires further investigation with respect to APOE allelic status. Further elucidation of the underlying mechanisms may provide insights for the development of treatments that might slow, arrest, or even reverse the development and progression of AD in at least a proportion of patients. The multiple mechanisms whereby apoE4 may influence the pathogenesis of AD suggest that it should be a high-priority target for drug development. Therapeutic approaches might include inducing the expression of apoE, recombinant apoE3 protein associated with liposomes, small molecules that can convert apoE4 to an apoE3-like molecule or abolish the adverse effects of apoE4, and gene therapy. Abolition of the influence of apoE4, and/or of factors that modulate its influence, on the pathogenesis of AD have great potential to delay the onset of the disease. As the populations of many developed nations age, AD will become an enormous public health problem. Interventions that could delay disease onset even modestly would have a major public health impact. ■

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