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FEATURE REVIEW

Apolipoprotein E, cholesterol metabolism, diabetes, and the convergence of risk factors for Alzheimer's disease and cardiovascular disease

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High fat diets and sedentary lifestyles are becoming major concerns for Western countries. They have led to a growing incidence of obesity, dyslipidemia, high blood pressure, and a condition known as the insulin-resistance syndrome or metabolic syndrome. These health conditions are well known to develop along with, or be precursors to atherosclerosis, cardiovascular disease, and diabetes. Recent studies have found that most of these disorders can also be linked to an increased risk of Alzheimer's disease (AD). To complicate matters, possession of one or more apolipoprotein E ε4 (APOE ε4) alleles further increases the risk or severity of many of these conditions, including AD. ApoE has roles in cholesterol metabolism and Aβ clearance, both of which are thought to be significant in AD pathogenesis. The apparent inadequacies of ApoE ε4 in these roles may explain the increased risk of AD in subjects carrying one or more APOE ε4 alleles. This review describes some of the physiological and biochemical changes that the above conditions cause, and how they are related to the risk of AD. A diversity of topics is covered, including cholesterol metabolism, glucose regulation, diabetes, insulin, ApoE function, amyloid precursor protein metabolism, and in particular their relevance to AD. It can be seen that abnormal lipid, cholesterol and glucose metabolism are consistently indicated as central in the pathophysiology, and possibly the pathogenesis of AD. As diagnosis of mild cognitive impairment and early AD are becoming more reliable, and as evidence is accumulating that health conditions such as diabetes, obesity, and coronary artery disease are risk factors for AD, appropriate changes to diets and lifestyles will likely reduce AD risk, and also improve the prognosis for people already suffering from such conditions.
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Introduction

Age-related diseases are becoming a major concern, especially in Western countries, as their populations grow older owing to advances in medical technology, pharmaceutical drugs, immunization, and health services. Dementia accounts for a large proportion of age-related diseases, and Alzheimer's disease (AD) is the most common form of age-related dementia. The social and economic consequences of this neurodegenerative disease present a significant challenge to society and it is imperative that strategies to prevent or delay the onset of AD are developed.[1]

In Australia, the number of people who suffer from AD is an estimated 135 000,[2] with this figure predicted to double by the year 2030.[3] In the US, age-adjusted death rates are on the increase for AD yet are decreasing for heart disease, cancer, and stroke.[3] About 12% of the total population over 65 years of age will develop AD in the US.[4] Over 80 years of age, this figure rises to 45%.[4] AD is a neurodegenerative disease which presents clinically with key symptoms

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including a progressive decline in memory, impairments in speech, language, spatial orientation, and dysfunction in the sensori-motor systems.[5–7] These symptoms subsequently result in an inability to cope with the demands of ordinary daily living, eventually leading to complete reliance on nursing care. Sufferers of AD are arbitrarily divided into two groups based on age of onset. Sufferers older than 65 years are classified as suffering from late-onset AD (LOAD), whereas those younger than 65 years are classified as early-onset AD (EOAD)[8] with a significant proportion of these cases exhibiting a family history and termed early-onset familial AD (EOFAD). However, the two groups have very similar neuropathological features. EOFAD is caused by autosomal dominant inheritance of mutations in the amyloid precursor protein (APP) or presenilin genes[9–14] in 50% of families, whereas the defective gene(s) in the remainder have yet to be identified. Although EOFAD cases only represent about 5% of all AD patients, studying the EOFAD defective genes has provided considerable insight into AD molecular pathology.[15] The majority of AD cases are late-onset cases, and are not thought to be due to genetic mutations. A combination of factors, including oxidative stress, abnormal lipid metabolism, abnormal glucose metabolism, physical inactivity, and cerebral hypoperfusion are thought to be necessary co-contributors or initiators of the disease process in these cases.

The memory loss and neurodegenerative damage of AD are essentially irreversible. Therefore, clinical research has been increasing emphasis on early diagnosis, and on the identification of risk factors that may be modified at preclinical or early clinical stages of the disease. Risk factors for LOAD that have been known for a while include old age, a family history of dementia, and possession of one or more APOE ε4 alleles.[16] The discovery of AD neuropathology in a large proportion of non-demented coronary artery disease (CAD) cases at post-mortem led researchers to investigate CAD as a risk factor. High cholesterol and CAD[17] are now believed to be important risk factors for AD. Other studies have shown that obesity,[18,19] type II diabetes mellitus,[20,21] and polymorphisms in the gene that codes for low-density lipoprotein receptor-related protein-1 (LRP-1) are also associated with LOAD.[22–24] Some studies suggest repeated head trauma is another risk factor for AD.[16,25,26] Risk factors for which there are conflicting results include gender and ethnic group.[27,28]

High cholesterol levels, obesity, diabetes, CAD, LRP-1, and apoE are physiologically or biochemically connected: they are all associated in some way with the transport and metabolism of lipids. This review brings together a lot of the evidence that has led to the above factors and conditions being linked to AD. It becomes obvious that it is very difficult to assess these factors in isolation owing to their biochemical connections, and it is likely that a combination of several of these factors add up to produce pathophysiological conditions liable to lead to AD.

Alzheimer's disease characteristics

AD is characterized histologically by the presence of intracellular and extracellular amyloid deposits in the brain, together with widespread neuronal cell loss. Extracellular amyloid deposits are known as neuritic or senile plaques.[29] Amyloid deposits within and around blood vessels and intracellular neurofibrillary tangles (NFT) are characteristics of AD brain pathology too, however these can occur in several other neurological disorders. The main protein constituent of AD senile plaques, a peptide known as Aβ[29,30] is a normal proteolytic product of a much larger transmembrane protein, the amyloid precursor protein (APP).[31] Aβ can be detected in plasma, cerebrospinal fluid (CSF), and in cell culture media.[32,33] APP can be cleaved by three proteases, classified as α, β, and γ secretases.[34,35] The protease α-secretase cleaves APP within the Aβ domain thereby precluding its formation. Aβ is produced via a two-step process involving the β-secretase or β-amyloid cleavage enzyme-1 (BACE-1)[34] and γ-secretase.[35] The two major forms of Aβ are Aβ1–40 and Aβ1–42 corresponding to 40 and 42 amino acid-long peptides, respectively. Aβ1–40 is synthesized in the early secretory and endocytic cellular pathways and Aβ1–42 is generated mainly in the secretory pathway.[36] In AD, Aβ peptides aggregate into insoluble fibrils which deposit in the brain to produce the characteristic amyloid plaques. Studies of the FAD-associated genetic mutations have shown them to lead to increased production of Aβ, particularly the longer, more amyloidogenic form Aβ1–42.[15] These studies as well as many *in vitro* and transgenic mice studies have led to the 'amyloid hypothesis', which states that Aβ accumulation is central to AD pathogenesis.[15] Although plaques and NFT are the obvious pathology of the disease, evidence suggests that plaques are large, almost inert 'tombstone' accumulations, and the 'toxic principle' of AD may consist of Aβ dimers or small soluble oligomers of the peptide.[37] AD is thought to be a central nervous system (CNS) disorder, however NFT-like tangles have been detected in the liver, pancreas, ovary, testis, and thyroid of AD patients, suggesting that AD may be a systemic disorder.[38]

Cholesterol and CAD as AD risk factors

In a 1990 study, abundant amyloid plaques were found in the brains of non-demented CAD subjects (75%), when compared to the brains of non-heart disease subjects (12%).[17] It is now recognized that individuals with heart disease often have demonstrable AD-like Aβ deposits within neurons in the brain,[39] and that cerebral atherosclerosis is strongly associated with an increased frequency of neuritic plaques.[40] As CAD has been linked to changes in cholesterol profiles, studies have tried to characterize the relationships between cholesterol metabolism, CAD and AD. Comparisons of total brain cholesterol

or plasma cholesterol levels in AD subjects relative to controls produced conflicting results.[39,41,42] However, more detailed studies found significantly lower plasma high-density lipoprotein (HDL) cholesterol levels and higher low-density lipoprotein (LDL) cholesterol levels in AD patients when compared to age-matched controls.[42,43,44] Similarly, serum apo A1 levels have been shown to be (a) markedly lower in AD patients and (b) highly correlated with AD cognitive decline.[44] Interestingly, decreased HDL cholesterol levels, increased LDL cholesterol levels, and decreased apo A1 levels are also known to be important risk factors for coronary atherosclerosis,[45] demonstrating an overlap in the risk profiles for the two conditions.

Cholesterol and AD pathogenesis

Aβ, apoE, cholesterol, and cholesterol oxidase have been shown to co-localize in the core of fibrillar plaques in transgenic mice models of AD,[46,47] supporting the suggestion that cholesterol and apoE are involved in fibrillar plaque formation.[47] Cholesterol may be directly involved in Aβ aggregation: abnormal oxidative metabolites such as cholesterol-derived aldehydes can modify Aβ, firstly promoting Schiff base formation, then accelerating the early stages of amyloidogenesis.[46,48] Other studies have shown that a novel Aβ species, having a conformation distinct from that of soluble Aβ, is characterized by its tight binding to GM1 ganglioside (GM1). This binding appears to be facilitated in cholesterol-rich environments and is dependent on the cholesterol-induced clustering of GM1 in the membranes.[46]

The changes in HDL- and LDL-cholesterol levels in AD suggest a disturbed cholesterol metabolism in AD. The cholesterol metabolite 24*S*-hydroxycholesterol is more soluble than cholesterol, and is more easily exported from the brain (Figure 1).[49] The amount of 24*S*-hydroxycholesterol exiting the brain is thought to reflect brain cholesterol synthesis levels, and CSF 24*S*-hydroxycholesterol levels are higher in AD individuals when compared with appropriate controls.[50–53] However, in severe cases of AD, plasma 24*S*-hydroxycholesterol/cholesterol ratios have been found to be reduced.[53] Cholesterol is converted to 24*S*-hydroxycholesterol by cholesterol 24-hydroxylase encoded by the CYP46 gene,[49] and it has been suggested that its levels may play a role in AD.[50–53] Some studies have found CYP46 gene polymorphisms are associated with AD pathophysiology,[54] however others have found CYP46 polymorphisms not to affect AD risk.[55,56]

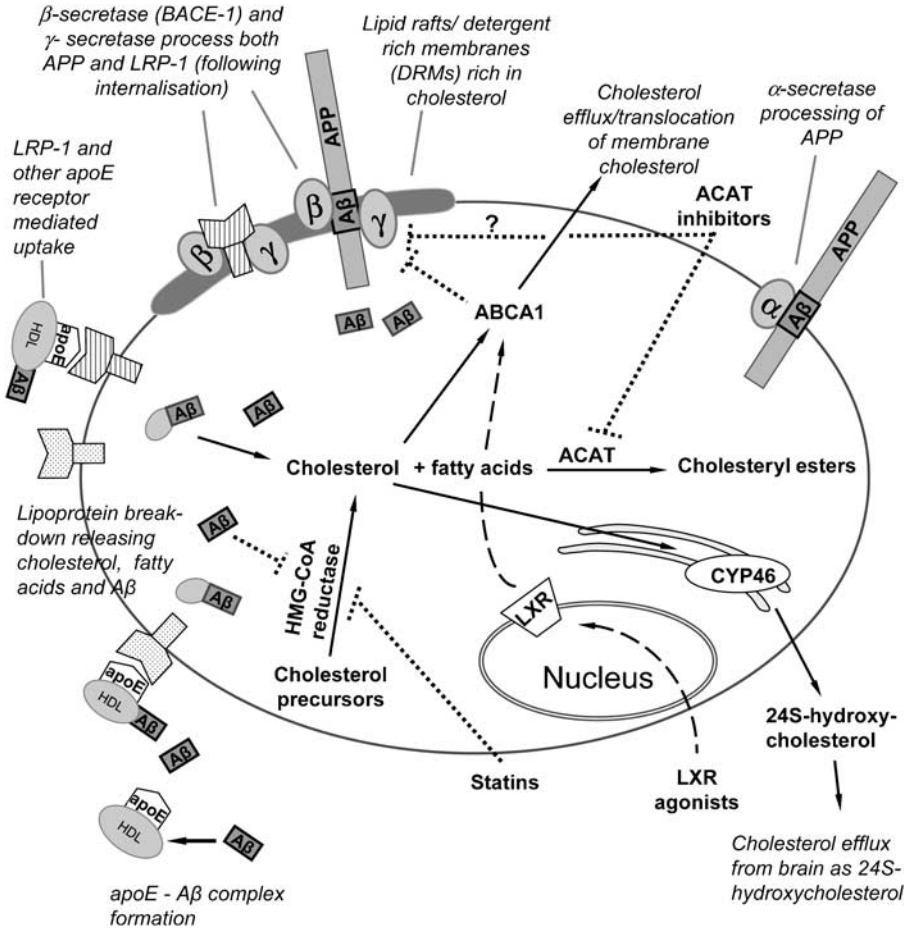

Figure 1 Some aspects of the interactions between cholesterol and APP metabolism.

In AD patients, the cholesterol flux is elevated.[57] The ATP-binding cassette transporter (ABCA1) is a major regulator of plasma HDL: it transports cellular cholesterol and phospholipids from cells onto HDL. ABCA1 plays a rate-limiting role in the process by which peripheral cholesterol is transported back to the liver for metabolism and subsequent excretion.[58] It has been hypothesized that if ABCA1 also stimulates cholesterol flux from the CNS to the periphery, it is likely to cause an increase in the internal cycling of brain cholesterol, and thereby prevent the accumulation of excess cholesterol in neurons (Figure 1).[59] In fact, ABCA1 has been detected in neurons, and increased expression is accompanied by cholesterol efflux from neurons and glia.[60] Increased neuronal expression of ABCA1 also affects APP processing, causing a decrease in Aβ production.[60] In a recent study, astrocyte cultures from ABCA1 (−/−) mice were found to secrete lipoprotein particles containing markedly less apoE and cholesterol, and had smaller apoE-containing particles than astrocyte cultures from ABCA1 (+/+) mice. The ABCA1 (−/−) mice themselves had greatly decreased levels of apoE in both the cortex (80% less than normal) and CSF (98% less than normal).[61] Individuals with a genetic polymorphism (R219K) in the ABCA1 gene have 30% lower cholesterol in their cerebrospinal fluid,[62] this polymorphism is therefore likely to modify brain cholesterol metabolism. Interestingly, this polymorphism is associated with a 1.7 year delay in AD age of onset.[62]

Cholesterol levels are also regulated by sterol regulatory element-binding proteins (SREBP). SREBP belong to a family of transcription factors that regulate intracellular cholesterol and lipid metabolism.[63] Factors SREBP-1a and SREBP-1c affect genes involved in fatty acid synthesis,[64] and a single G deletion (ΔG) polymorphism in the SREBP-1a gene has been found to lower 24*S*-hydroxycholesterol levels, increase clearance of cholesterol from the brain, and thereby reduce brain cholesterol levels.[65] In APOE ε4 carriers, this polymorphism is associated with a decreased risk of AD, underscoring the significance of abnormal cholesterol metabolism in AD.[65]

Cholesterol modulates the expression of APP and Aβ

The intracellular metabolism and distribution of cholesterol markedly affects APP metabolism.[66] Cell culture studies have found that APP inside cholesterol-rich cell membrane lipid raft clusters (detergent-resistant membranes, or DRMs) is cleaved by BACE-1, whereas APP outside rafts undergoes cleavage by α-secretase[67] (Figure 1). The completely assembled, biologically active γ-secretase complex also resides within DRMs.[68] Studies have shown that lowering membrane cholesterol (e.g. by statin treatment) causes both an increase in the secretion of soluble APP (sAPP, α-secretase product) and a decrease in Aβ production.[67,69–71] Increasing membrane cholesterol decreases the secretion of sAPP,[72,73] possibly due to cholesterol interfering with glycosylation in the protein secretory pathway.[74] As sAPP is thought to have neurotrophic properties, decreasing sAPP levels may promote neurodegeneration. However, in another study, the *significant* reduction in membrane cholesterol of hippocampal membranes from AD patients and rodent hippocampal neurons again caused a decrease in Aβ production, yet a more *moderate* reduction in membrane cholesterol in this latter study caused an increase in Aβ production, suggesting a dose-dependent effect.[75]

The links between cholesterol and APP metabolism add up to a complex web of interactions (some of which are depicted in Figure 1). LRP-1, a receptor likely to be involved in brain lipid and cholesterol distribution, binds and internalizes a diverse array of ligands including Aβ–ApoE complexes in liver cells as well as some forms of sAPP.[76,77] This receptor has been shown to be another substrate for BACE-1.[78] LRP-1 is also a γ-secretase substrate: it interacts with PS-1, a critical component of γ-secretase, and it competes with APP for γ-secretase activity.[79] In other studies, Aβ1−40 has been found to reduce cholesterol synthesis by inhibiting the enzyme 3-hydroxy-3 methylglutaryl coenzyme A (HMG-CoA) reductase (involved in cholesterol synthesis), in a putative negative feedback loop, and Aβ1−42 has been found to activate neutral sphingomyelinase, an enzyme that cleaves sphingomyelins to produce ceramides, thought to be primary effectors of apoptosis.[80] This latter finding is consistent with the fact that ceramide:sphingomyelin ratios are higher in vulnerable brain regions of AD patients.[81]

The liver X receptors (LXR) are nuclear receptors that induce a variety of genes involved in cellular cholesterol efflux and are expressed in the cells of the CNS.[82] A major LXR target gene in the brain has been shown to be ABCA1 (Figure 1).[83] Activation of LXR has been shown to stimulate ABCA1 levels and decrease Aβ concentrations.[83] Therefore, LXR activation may provide a novel approach for the treatment of AD.

Cholesterol esterification, APP metabolism, and AD

The enzyme acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT) esterifies cholesterol with long-chain fatty acids, and thereby controls the ratio of cellular free cholesterol and cholesterol esters.[84] Cholesteryl ester (CE) levels appear to have a profound effect on APP processing, as ACAT inhibitors directly modulate Aβ generation, and in cells that lack ACAT activity, production of Aβ is completely abolished (Figure 1).[85,86] In transgenic mice expressing human APP, treatment with the ACAT inhibitor (CP-113 818) decreased brain CE and amyloid pathology, and resulted in an improvement in spatial learning.[87] A polymorphism in the ACAT gene (the A/A genotype of rs1044925) that results in low brain cholesterol content has been associated with low brain amyloid load and a reduced risk for AD in European populations.[88] ACAT inhibitors have been widely studied for

the treatment of atherosclerosis, and may prove equally important for the treatment and prevention of AD. However, the exact mechanism by which CE levels modulate the generation of Aβ is not known.

Statins and AD

Large-scale studies have indicated that certain cholesterol-lowering drugs (statins) reduce the risk of AD in human subjects,[89,90] therefore they are currently being tested as potential therapies for the prevention of AD. Statins are inhibitors of the enzyme HMG-CoA reductase (Figure 1), the rate-limiting enzyme involved in cholesterol synthesis. Statins also trigger an upregulation of LDL receptor levels, which in turn assists in reducing LDL levels.[89,90] In some studies of transgenic mice and guinea pigs, cholesterol-lowering agents have been found to reduce plasma and brain cholesterol;[91–93] however, in other guinea pig studies, despite an 83% reduction in plasma cholesterol, brain cholesterol content was unaffected by simvastatin or pravastatin.[94,95] Many (but not all) studies suggest that lowering cholesterol levels causes a decrease in Aβ levels, for example, in the latter study levels of Aβ1–42 and Aβ1–40 in the brain and CSF of guinea pigs were reduced by simvastatin.[95] In contrast, one study using AD model transgenic mice found that lovastatin decreased cholesterol levels in all the mice, yet enhanced Aβ production, although only in the female mice.[93] A recent clinical study has found that statin treatment over 12 months had no effect on patient Aβ levels in plasma or CSF, yet there was evidence that the non-amyloidogenic pathway was enhanced.[96]

The mechanisms of action of statins in lowering the risk of AD are evidently still unclear: one suggestion has been that the improved brain oxygenation resulting from statin treatment is more relevant in reducing AD risk[97] and in fact recent results of the Rotterdam study suggest that cerebral hypoperfusion precedes and possibly contributes to the onset of clinical dementia.[98] Other non-statin cholesterol lowering drugs have also been tested, and one such drug, probucol, can lead to significant increases in CSF apoE levels and a decrease in CSF Aβ1−42 in mild to moderate sporadic AD.[99] Interestingly, the recent finding that Aβ1−40 peptides can inhibit HMG-CoA reductase in a putative negative feedback loop is consistent with the theory that reducing cholesterol production/levels will lower γ-secretase activity.[80]

Statins have also been shown to increase lipoprotein lipase (LPL) activity. LPL is an enzyme that hydrolyses triglyceride-rich lipoproteins (chylomicrons and very-low-density lipoproteins (VLDLs)) to convert triglycerides to fatty acids and glycerol. After hydrolysis of triglyceride-rich particles by LPL, cholesterol-rich remnants are formed that are cleared mainly by the liver following receptor-mediated endocytosis. In the brain, LPL binds to apoE particles and LRP-1 for the transfer of lipids to cells. LPL has been found in amyloid plaques in AD individuals, and mutations in the LPL gene have been associated with clinically diagnosed AD, suggesting that inadequate levels of LPL increase the risk of the disease.[100] In contrast, the LPL 447ter allele has been associated with a protective effect on AD, resulting in fewer amyloid plaques and tangles, and less neuronal death when compared with AD patients lacking this allele.[100] Rabbit and rat studies have shown that statin treatment can markedly increase LPL activity, decrease plasma triglyceride levels, and increase LPL mRNA expression in certain tissues.[101,102] Therefore, it would appear that statins can favourably modify lipid metabolism in several ways, and more studies are needed to establish how statins decrease the risk of AD.

High fat/high cholesterol diets, obesity, and AD

Epidemiological studies have shown that people of similar ethnic origins yet living in different environments can have significantly different risks of dementia and AD.[103,104] For example, Nigerians in Africa have a much lower incidence of AD when compared with African Americans living in the US.[103] Similar results were obtained with Japanese people living in Japan: they have much lower rates of AD when compared with Japanese Americans living in the US.[104] Diet and fat intake appear to be important when comparing the lifestyles of populations screened for AD,[103] however epidemiological results have produced conflicting results. For example, one study has found that the more fat consumed in a meal, the greater the risk of developing AD, and senile dementia, more generally.[41] However in the large Rotterdam study, a high total intake of saturated fat and cholesterol was not associated with an increased risk of dementia.[105] Fat consumption is only one aspect of diet, and other environmental factors and lifestyle issues such as level of exercise, oxidative stress, and other aspects of diet may be of equal importance when considering AD risk.

Animal studies have produced less conflicting results, possibly as it is easier to eliminate unwanted variables. A strong correlation between high fat/high cholesterol diets and increased brain Aβ levels has been shown in numerous experimental animal models, demonstrating that an inappropriate diet is likely to increase the risk of AD in the same way it increases the risk of CAD and stroke. For example, in rabbits with diet-induced hypercholesterolemia, increased levels of Aβ and apoE protein have been found in the temporal and frontal cortex of the brain,[106] and in Watanabe rabbits with a genetic defect in the LDL receptor, both hypercholesterolemia and neuronal Aβ deposition occur.[107] AD transgenic mouse models develop Aβ plaque-like deposits more quickly if fed a high fat /high cholesterol diet,[108] and the levels of brain Aβ in these mice correlate strongly with both plasma and CNS total cholesterol levels.[108] Caloric restriction, in contrast, decreases Aβ peptide generation and neuritic plaque deposition in the brains of such mouse models.[109] Interestingly, guinea pigs fed a high cholesterol diet show increases in plasma but not brain cholesterol levels. Instead, *de novo*

cholesterol synthesis appears to be inhibited, as there is a decrease in the levels of the cholesterol precursor lathosterol.[110]

The conflicting results in the human epidemiological studies mentioned above with respect to fat intake may also have arisen from differences in the types of dietary fat. For example, studies in humans and other animals have reported effects of high fat intake on cognitive performance; with saturated and polyunsaturated fats having apparently opposite effects. Animal studies have demonstrated that feeding high levels of saturated fat to rats for 3 months can result in severe learning and memory impairments,[111–114] yet diets with chronically high levels of polyunsaturated fatty acids result in better discrimination learning than those diets containing saturated fat.[113]

Many clinical studies, as well animal studies such as those mentioned above, have shown that high fat/high cholesterol diets lead to increases in brain Aβ levels and to HDL/cholesterol and LDL/cholesterol levels linked to AD and CAD, therefore it is understandable that obesity is now also recognized as an important risk factor for AD.[115–117] Obese men have been found to have lower cognitive function when compared with non-obese normotensive men,[115] and in an 18 year follow-up study in overweight women, a high risk for dementia (particularly AD) was found in these women relative to controls.[116] In our own recent studies, we have observed a strong positive correlation between body fat and blood Aβ1−42 levels in cognitively normal individuals,[118] and in a recent longitudinal study of 1149 individuals, mid-life obesity was found to be a significant risk factor for AD later in life.[119] This association has now also been linked to hyperinsulinemia: insulin infusion (inducing temporary hyperinsulinemia) increases blood plasma Aβ1−42 levels in cognitively normal individuals who are offspring of AD patients: and the magnitude of Aβ1−42 increase is greater in subjects with increased abdominal body fat.[117]

High fat diets appear to interfere with glucose tolerance and insulin sensitivity, and again have different effects depending on the types of fat.[120–122] The risk of type II diabetes is also associated with a high trans-fatty acid intake and a low unsaturated: saturated fat intake ratio.[123] There are reports that saturated and trans-fatty acids increase insulin resistance, whereas mono- and poly-unsaturated fats decrease resistance and offer protection against disease.[123–125] Therefore, the detrimental effect of a prolonged high fat diet on cognitive performance may, at least in part, be due to abnormalities in glucose regulatory mechanisms.

Glucose regulation, insulin resistance, diabetes, and AD

The insulin-resistance syndrome is characterized by insulin resistance, hyperinsulinemia, impaired glucose tolerance, abnormal cholesterol and/or triglyceride levels, low HDL, high blood pressure, and obesity, also all independent risk factors for CAD. Insulin-resistance syndrome is a precursor to type II diabetes. Many studies have now associated insulin-resistance syndrome and diabetes with AD,[20,126–129] and diabetes-linked cerebrovascular disease has been suggested as the connection,[20,127,128] however, abnormal glucose regulation itself may be of equal importance, as discussed below. Type II diabetes is also associated with an increased risk for vascular dementia.[20,128,130–132] In obese and diabetic individuals, there is a marked decrease in the clearance and metabolism of cholesterol-rich lipoproteins from plasma.[133] Cholesterol synthesis is also elevated and cholesterol absorption impaired in diabetes,[134,135] and this is thought to be related to impaired blood glucose regulation.[135]

The association between diabetes and AD is particularly strong among APOE ε4 carriers: individuals with type II diabetes who possess the APOE ε4 allele have twice the risk of developing AD as compared with non-diabetics with APOE ε4.[136] In addition, brain pathology from type II diabetic patients frequently includes amyloid deposition or NFT, yet amyloid deposition is markedly greater in individuals with both diabetes and the APOE ε4 genotype.[136,137]

Glucose metabolism and brain function in AD

Studies using positron emission tomography (PET) have consistently documented decreased brain glucose metabolism in moderately and severely demented patients compared to age-matched normal individuals,[138,139] and recent studies have shown impaired glucose utilization in neocortical association areas of the brains of patients with mild cognitive deficit, a precursor to AD.[140] In diabetic individuals, therefore, impairments in glucose and insulin regulation may contribute to AD pathology through mechanisms including decreased cortical glucose utilization, particularly in the hippocampus and entorhinal cortex. Studies have in fact found a correlation between blood glucose levels and memory performance in AD patients,[138,141] although in the fasting state, others have observed no differences in resting glucose and insulin levels in AD patients compared to age-matched controls.[132]

Findings after oral glucose testing have varied, with the following all being observed in AD patients relative to controls: (i) higher insulin levels,[142,143] (ii) higher glucose levels,[141] (iii) lower glucose levels,[142] and no differences between patients and controls.[144,145] However, at the time of these studies, the relevance of excluding patients with cerebrovascular disease or diabetes was not known, and accurate diagnostic techniques for early stages of AD were not available. Therefore patients with one or more of these conditions may have been inadvertently included/wrongly categorized in some of these studies, confounding the results.[132] The stage of AD is also of importance, as high insulin levels are observable at the mild stages of AD, but decline as dementia progresses.[145]

Paragraph recall memory tasks are known to be sensitive indicators of mild AD, and these have been used to demonstrate improved cognitive functioning in AD patients following glucose ingestion.[141] AD patients display a greater increase in blood glucose levels after peripheral glucose administration than controls, supporting the hypothesis of a systemic dysfunction in glucose regulation in AD.[146,147]

A longitudinal investigation using intravenous glucose administration[148] found that memory performance (immediate and delayed paragraph recall) improved in the hyperglycaemic stage for patients with very mild AD, and at follow-up (12–18 months later), very mild AD patients who had remained at this stage of dementia again showed significant hyperglycaemic memory facilitation. In contrast, very mild AD patients whose dementia had progressed showed no hyperglycaemic enhancement of memory performance at follow-up. Therefore, these researchers suggested that the degree of cognitive facilitation during hyperglycaemia may be of prognostic relevance regarding the severity and progression of AD. However, other researchers have found that the administration of glucose (50 g) to moderately to severely demented patients (probable AD) did improve memory.[149]

Deficits in glucose metabolism might also potentiate the neuronal cell death produced by other pathological processes (such as cerebral hypoperfusion, abnormal cholesterol metabolism, or high levels of toxic Aβ), which in turn might be influenced by genetic predisposition such as possession of APOE ε4 alleles. In support of this argument it has been demonstrated that glucose utilization in the brain is reduced in younger (47–68 year old) asymptomatic individuals who carry an APOE ε4 allele, when compared to non-APOE ε4 individuals.[150] Glucose regulatory mechanisms can also affect APP metabolism: following ingestion of glucose, blood insulin and glucose levels significantly increase, whereas plasma APP concentration decreases.[151]

Insulin, insulin receptors, and Aβ peptides

A link between insulin-resistance syndrome or diabetes and AD has been debated for over a decade, and most evidence now supports the theory that these are risk factors for AD. Several molecular mechanisms apart from the abnormal glucose regulation mentioned above and diabetes-associated cerebrovascular disease have been proposed to be responsible for this increased risk, and the true picture may be a combination of all these mechanisms. For example, the formation and accumulation of advanced glycation end products (AGEs) occurs in diabetes, and recent studies have confirmed that AGEs, and interactions with their receptor (RAGE), may play a role in the pathogenesis of diabetic vascular complications and neurodegenerative disorders including AD. AGEs have been detected in both AD plaques and NFTs, and glycation of Aβ enhances its aggregation *in vitro*. RAGE has also been found to be a cell surface receptor for Aβ, eliciting neuronal cell perturbation.[152]

Insulin and/or insulin receptors appear to contribute to learning and memory via the activation of specific signalling pathways, one of which is associated with long-term memory formation,[153] therefore, desensitization of the neuronal insulin receptor, which occurs in diabetes and the insulin-resistance syndrome, may be another key factor in the pathogenesis of AD.[147]

Insulin levels may also have an impact on the regulation of Aβ proteolytic degradation, as the insulin degrading enzyme (IDE) can break down several peptides, including insulin, Aβ, glucagon, and amylin. A recent study has shown that the APP intracellular domain (AICD) can also be cleaved by IDE.[154] In APP transgenic mice lacking the IDE gene, there is a 50% decrease in amyloid degradation, an increase in AICD fragment levels, and brain amyloid accumulation.[155] IDE (−/−) mice also develop hyperinsulinemia and glucose intolerance. It has been suggested that when insulin levels are elevated in diabetes, IDE preferentially degrades insulin leading to higher levels of Aβ.

Diet-induced insulin resistance in AD-model transgenic mice promotes amyloidogenic Aβ generation in the brain, due to increased γ-secretase activities and decreased IDE activities, and recent clinical studies have shown that induced hyperinsulinemia causes an increase in plasma and brain Aβ1−42 levels, as well as increased levels of inflammation markers in CSF.[156] Insulin resistance also leads to a functional decrease in insulin receptor (IR)-mediated signal transduction in the brain, again consistent with the hypothesis that hyperinsulinemia[157] or insulin resistance[158] may potentiate the risk of AD. A pathological feedback mechanism may occur between increased Aβ generation and high insulin levels characteristic of insulin resistance, accelerating the pathological process. In support of this, we have demonstrated that elevated Aβ levels, characteristic of AD, inhibit insulin binding to the insulin receptor.[159] In fact, Aβ appears to be a direct competitive inhibitor of insulin binding to its receptor.[160] One of the many effects of insulin binding to the insulin receptor is the promotion of sAPP secretion, and Aβ binding to insulin receptors can inhibit this effect.[160] Inhibitor studies have implicated the phosphatidyl inositol 3 kinase (PI3K) signalling pathway in the promotion of sAPP secretion by insulin.[161] Adding further links between insulin and APP metabolism are other recent studies which have found that insulin also reduces intracellular accumulation of Aβ, by accelerating APP trafficking through the trans-Golgi network (a major site for Aβ production) to the plasma membrane. In this latter study, insulin's effect on APP metabolism was found to be mediated via a receptor tyrosine kinase/mitogen-activated protein kinase (MAPK) kinase pathway.[162]

Recent studies have linked a polymorphism in the MAPK81P1 gene with AD.[163] This gene codes for islet-brain1 (IB1, DNA-binding transactivator of the glucose transporter GLUT2)/c-Jun N-terminal kinase

interacting protein-1 (JIP-1), a neuronal scaffold protein that interacts with several membrane proteins including LRP, ApoE $\varepsilon 2$, the reelin receptor, and APP. The $-499A>G$ promoter polymorphism in MAPK81P1 causes an increase in transcription, resulting in enhanced binding activity. This polymorphism was not found to be linked to AD in the general population, however a strong association was found in subjects also carrying a particular LRP gene polymorphism (CC genotype).[163] A separate polymorphism in the MAPK81P1 gene has been linked with diabetes.[164] A missense mutation in the coding region of this gene segregated with diabetes in a diabetes type II family. *In vitro* studies found that this mutation reduced insulin transcription, and reduced IB1's ability to prevent apoptosis.[164]

Insulin-like growth factors, their receptors, and changes in AD

The insulin-like growth factors IGF-I and IGF-II exert a variety of effects on cell metabolism, cell proliferation, apoptosis, and differentiation.[165] For example, as well as promoting glucose utilization, IGF-1 promotes neuronal survival during brain development, projection neuron growth, dendritic arborization, and synaptogenesis.[165,166] IGF receptors include the insulin receptor, the type 1 IGF receptor (IGF-IR), and the type 2 IGF receptor (IGF-IIR)/mannose-6-phosphate (M6P) receptor, thereby activating some form of signalling.[165] IGF-IR and the insulin receptor belong to the subfamily of receptor tyrosine kinases, yet mediate different effects.[167] High levels of IGF-1 and IGF-1 receptors are expressed in the brain, particularly in the hippocampus.[168]

Transcription of the genes for IGF-I, IGF-II, and the insulin and IGF-I receptors is reduced in AD brains when compared to controls.[169] AD brains also demonstrate reduced expression of the insulin receptor substrate (IRS), IRS-associated PI3K, and activated Akt/protein kinase B; suggesting downstream abnormalities in the insulin and IGF intracellular signalling mechanisms in AD brains.[169] As mentioned earlier, activation or inhibition of these signalling mechanisms can influence the metabolism of APP and Aβ.[161,162]

IGF-1 has also been shown to block Aβ toxicity in primary cultures of hippocampal neurons.[170] This inhibition of Aβ toxicity occurs by activation of extracellular signal-regulated kinase (ERK), activation of Akt/protein kinase B, and the prevention of c-Jun N-terminal kinase (JNK) activation in a PI3K-dependent manner.[171,172] Conversely, secreted APP, which is known to have neurotrophic properties, has been shown to stimulate the phosphorylation of IRS-1, thereby activating the IRS-1 signalling pathway.[173]

Apolipoprotein E function, alleles, and its role in cerebral cholesterol homeostasis

ApoE structure and isoforms

ApoE is a polypeptide of 299 amino acids with a molecular weight of 34 kDa.[174] It is highly ordered in terms of its physical structure, and apoE derived from the CSF is post-translationally different to that in plasma. Isoelectric focusing-based studies have revealed three main isoforms of apoE in humans,[175] and these arise from single amino-acid substitutions with arginine or cysteine at residues 112 and 158, resulting in significant functional differences.[176] Each isoform is coded for by the APOE gene on chromosome 19q13.2.[174,175] The most common allele in the human population is APOE $\varepsilon 3$, followed by APOE $\varepsilon 4$ and APOE $\varepsilon 2$.[177] For example, in the Australian population, the $\varepsilon 3$, $\varepsilon 4$, and $\varepsilon 2$ allele frequencies are 78, 14, and 8%, respectively.[176] Most studies conducted on other populations agree that the order of allele frequency is $\varepsilon 3 > \varepsilon 4 > \varepsilon 2$, even if the frequencies are not identical between various populations.[178–180]

ApoE function

In the periphery, apoE aids the transport of triglyceride, phospholipid, cholesteryl esters, and cholesterol into cells, by mediating the binding, internalization, and catabolism of lipoprotein particles.[181] It is the main ligand for the LDL receptor found on the liver and other tissues, and for the specific apoE receptor (chylomicron remnant) of hepatic tissues.[181] In human CSF, most of the apolipoprotein content is represented by apoE and apoA and these are present on astrocyte-secreted lipoproteins which have a density similar to plasma HDL.[182,183] ApoE is required for lipoprotein uptake via LDL receptors and LRP-1 in the CNS, most likely in order to mediate the uptake and redistribution of lipids and cholesterol within the CNS, as it does in the periphery.[183–186]

Cholesterol homeostasis and ApoE

Cholesterol can be synthesized in the brain, therefore brain cholesterol homeostasis may be independent of the periphery.[187] In support of this, dietary levels of cholesterol have marked effects on *de novo* peripheral cholesterol synthesis, yet appear to have little or no effect on brain cholesterol synthesis or metabolism.[188] In addition, although one study has found that LDL can cross the blood-brain barrier (BBB) by receptor-mediated transcytosis,[189] most studies suggest that plasma lipoproteins do not cross the BBB.

In AD, brain cholesterol flux is elevated: when compared to controls, higher levels of the more soluble form of cholesterol, 24*S*-hydroxycholesterol, are found in both CSF and plasma of AD patients, even in early stages of dementia,[50–53] although the cause of this is unknown. As mentioned in an earlier section, AD patients respond positively to cholesterol-lowering drugs. This underscores the relevance of cholesterol metabolism in AD, despite the fact that brain cholesterol levels are not necessarily affected by the drugs.

ApoE is known to play a greater part in normal cholesterol metabolism than any other protein. For example, the various apoE isoforms interact differently with specific lipoprotein receptors, resulting in significantly different effects on cholesterol

metabolism.[181] ApoE appears to be required for cholesterol mobilization and lipid homeostasis in the CNS, and it is likely that CNS apoE levels, distribution, and ApoE allele status can influence AD risk via fluctuations in CNS cholesterol metabolism.[181]

ApoE and AD

In 1993 a locus within an apolipoprotein gene cluster on chromosome 19 was shown to be a risk factor for AD. ApoE was implicated, based on the knowledge that apoE is found in plaques and NFT, it binds the Aβ peptide, and the fact that it is also the commonest brain apolipoprotein. A strong allelic association between AD and the APOE ε4 allele was subsequently demonstrated.[190,191] However, this risk appears to be limited to Western countries: in less-developed countries, there is often no increased risk for AD associated with the APOE ε4 allele.[192,193] APOE allelic variation is also thought to influence one's risk for cardiovascular disease (CVD), particularly CAD, via its effect on cholesterol levels. APOE ε4 alleles are associated with higher total cholesterol and higher LDL cholesterol levels than average, whereas APOE ε2 alleles are associated with lower levels of these markers.[181] The higher intake of fat in the Western diet may be partly responsible for the increased risk of AD associated with APOE ε4, when compared with lower fat intake in less-developed countries.[194,195] In contrast, the APOE ε2 allele appears to protect against developing AD, as this allele is significantly under-represented in many populations of AD-affected individuals.

ApoE isoforms and synaptic plasticity/neurite outgrowth

A deficiency in either apoE or the LDL receptor in mice results in impaired learning and memory functions,[196,197] implying an important role for ApoE in synaptic plasticity. Recent studies have even found isoform differences: apoE ε4, when compared to apoE ε3, inhibits synaptic plasticity in the hippocampus and entorhinal cortex, following environmental stimulation.[198]

Cultures of rabbit dorsal root ganglia treated with apoE ε3-loaded lipoproteins show significantly greater neurite extension and branching than those treated with apoE ε4-loaded lipoproteins.[199] This effect requires the binding of apoE to LRP-1.[184] It has also been found that intraneuronal apoE interacts with the microtubule-associated proteins tau and MAP2, affecting microtubule formation, the polymerization of tubulin and thereby influencing neurite extension.[200] Studies using N2A cells[201] and primary cultures of mouse hippocampal neurons[202] found that incubation with apoE ε4 produced fewer polymerized microtubules than incubation with apoE ε3:[201] ApoE ε4 was found to destabilize microtubule assembly, whereas apoE ε3 stimulated the polymerization of β-tubulin and the formation of microtubules.[201]

ApoE also has a key role in the repair process after neuronal injury. In response to brain lesions, apoE mRNA levels increase, neuronal cholesterol synthesis decreases, and lipoprotein binding to the cell increases.[203–205] Collectively, these changes suggest that apoE helps to repair cells by recycling membrane components from damaged cells. As yet, few studies have examined apoE ε2 and its apparent protective effect in AD with respect to neuronal modelling and plasticity.

ApoE/Aβ interactions

ApoE has been found to be directly involved in APP metabolism. For example, apoE added to cell cultures causes a decrease in Aβ secretion and an accumulation of APP C-terminal fragments in cell extracts, suggesting an inhibition of γ-secretase.[206] ApoE has also been shown to bind to the N-terminus of APP (independent of Aβ region)[207] and this is thought to influence maturation and secretion of APP.[208] The ApoE receptor LRP-1 binds sAPP, the secreted form of APP, and mediates its degradation.[76] However, more studies have been carried out on the interactions between Aβ and apoE.

ApoE/Aβ complexes are major components of AD brain amyloid deposits,[209–211] and among AD patients, individuals homozygous for APOE ε4 manifest greater extracellular amyloid plaque size and density.[212,213] Further studies showed that ApoE can accelerate the transformation of soluble Aβ into the β-pleated sheet conformation of amyloid fibrils *in vitro*.[214] This led to the hypothesis that apoE may be involved in Aβ aggregation and plaque formation. Studies soon produced support for this theory, as it was found that purified apoE forms SDS-resistant complexes with Aβ, and purified apoE ε4 forms the most stable complexes, when compared to purified apoE ε3 or apoE ε2.[191,215,216] However, it was later discovered that the purification of apoE, involving delipidation and denaturation, alters the behaviour of apoE relative to the native biologically available form.[217,218] In fact, native non-denatured apoE ε2 and apoE ε3 bind Aβ avidly, while apoE ε4 has a much lower affinity for the peptide.[217,219,220] It was also found that apoE may be involved in Aβ clearance, as the binding of apoE to Aβ actually reduces Aβ toxicity in culture. LDL receptors were found to be necessary for this effect, suggesting LRP-1-mediated uptake and degradation of Aβ.[221] This apoE-mediated Aβ binding and uptake was shown to be promoted by apoE ε2 and apoE ε3 but not apoE ε4.[221,222] These latter findings led to the alternate hypotheses that apoE/Aβ complex formation promotes Aβ clearance via LRP-1-mediated uptake and degradation, and that apoE ε4 weakly associates with Aβ (compared to apoE ε2 or apoE ε3), indicating a reduced ability to clear Aβ, thereby promoting Aβ accumulation and senile plaque formation. It has been suggested that Aβ/ApoE binding alters the conformation of Aβ, and not only mediates cellular uptake via apoE receptors but it may also decrease neurotoxicity and neuroinflammation in the brain.[223]

Studies of peripheral Aβ clearance have shown similar patterns. For example, physiological levels of

Aβ reduce the binding of apoE ε3 or apoE ε4 lipoprotein-like emulsions to liver cells, yet apoE ε2 enhances the binding.[224] The apoE/Aβ interaction promotes the uptake of lipoproteins via the LRP-1 pathway in liver cells, a pathway likely to be vital in both cholesterol and Aβ metabolism,[224] and supporting this theory, our Aβ clearance studies in apoE (+/+) and apoE (−/−) mice have shown that the interaction of apoE with Aβ receptors for uptake by influences its clearance from the kidney and liver.[225] In other apoE studies, Aβ (at much higher concentrations) almost abolished the binding and uptake of apoE ε3 or apoE ε4-rabbit β-very-low-density lipoproteins (βVLDL) by fibroblast cells.[226]

ApoE and Aβ transport across the BBB

The transport of circulating Aβ into the brain has been detected in some animals, and another LDL receptor-related protein, LRP-2 (also known as megalin), has been identified as the BBB receptor involved in the uptake of apoE/Aβ complexes into the brain. *In vitro* studies indicate that other receptors, such as the receptor for AGEs (RAGE) and the macrophage scavenger receptor can mediate the binding of Aβ to the BBB and regulate uptake into the brain.[227] However, most transport of Aβ across the BBB appears to occur in the direction of brain to periphery, following the concentration gradient, and evidence suggests that this transport, followed by degradation in the liver, is the main mechanism for Aβ removal from the body as mentioned above.[225,227] Aβ transport across the BBB to the periphery has been shown to be mediated by LRP-1 and regulated by ligands such as apoE and α2-macroglobulin.[227] P-glycoprotein expressed on the BBB has also recently been shown to aid Aβ transport into the periphery,[228] providing a novel therapeutic target, as many drugs are known to enhance its activity. The importance of LRP-1 in Aβ efflux from the brain is underscored by recent studies that have shown the homeobox gene MEOX2 is expressed at very low levels in brain endothelial cells of AD autopsy samples, and that reducing MEOX2 expression at the BBB causes a significant reduction in both LRP-1 expression and BBB clearance of Aβ.[229] However it should be noted that it is not yet known if the drop in MEOX2 expression in AD precedes or is secondary to the neuronal loss found in AD.

ApoE's involvement in Aβ metabolism is not restricted to facilitating the removal and degradation of the peptide, either via brain cells or via transport across the BBB. The brains of aged APP^{V717F} AD transgenic mice contain considerable fibrillar amyloid deposits. Crossing these mice onto a mouse apoE knockout background results in a substantial reduction in Aβ load and almost no neuritic degeneration.[230] This suggests apoE is in fact a requirement for Aβ deposition, and supports the early studies that found ApoE can accelerate Aβ fibril formation.[214] However, when the AD apoE (−/−) transgenic mice carry human apoE isoforms, there is a reduction in amyloid load when compared to the original AD transgenic mice, with apoE ε3 being more effective than apoE ε4 at reducing this load.[230] Studies have yet to determine whether the role of apoE as a pathological chaperone outweighs its role in Aβ clearance, however these results reinforce the theory that apoE ε4 is not an effective mediator of Aβ homeostasis in the brain.[230]

Apolipoprotein E and sulphatide in AD

Sulphatides are sulphated galactocerebrosides, a subclass of sphingolipids produced mostly by oligodendrocytes in the CNS, and they mediate processes like cell growth, neuronal plasticity, and signal transduction. A deficiency in sulphatide and an increase in ceramide, a lipid second messenger thought to be a degradation product of sulphatide, occur very early in AD. In addition, significant reductions in CSF sulphatide levels occur in incipient AD, supporting the theory that disturbed lipid metabolism occurs early in AD pathogenesis, and potentially providing an excellent marker for AD.[231] A new explanation for apoE ε4's role in AD has also been provided, as transgenic mouse studies have shown that sulphatide levels are dependent on APOE allele status, with the lowest levels being found in apoE ε4 expressing mice.[232]

Conclusions

In Western countries, obesity and type II diabetes are becoming very common conditions. These are both known to be risk factors for atherosclerosis and other CVDs, and now also AD. The Western diet, known to be high in fat, particularly cholesterol, is known to increase considerably the risk of obesity and type II diabetes. As factors such as abnormal insulin regulation and abnormal cholesterol metabolism have been discovered in each of these conditions, overlap in pathogenesis has been suggested. In support of this, apoE ε4 does not appear to be as effective as apoE ε3 or apoE ε2 in the maintenance of cholesterol homeostasis, and possession of apoE ε4 alleles can increase the risk of most of these conditions as well.[137] The treatment of AD patients with cholesterol-lowering drugs such as statins, already proven effective in the treatment of CVD, has been associated with a reduced risk of AD.[49,94] This reduced risk may be associated with reduced brain cholesterol levels and reduced Aβ production, however improved brain oxygenation may be equally relevant. ApoE also appears to be intimately involved in Aβ degradation in the brain, Aβ clearance from the brain, Aβ deposition, neurite outgrowth, and sulphatide content. The relative importance of each of these roles in AD risk is not yet clear.[223,227]

Caloric restriction and exercise,[233,234] and diets with low fat content and high antioxidant, trace mineral, and fish content have been associated with a decreased risk of AD.[235,236] Possession of APOE ε4 alleles often does not increase the risk for AD in countries where people have low fat diets and more

active lifestyles, supporting the concept that modifiable lifestyle factors may contribute significantly to the risk of AD.

An assessment of AD risk that takes into account both environmental and genetic factors may well provide the most useful model for clinical management in the future, with the emphasis on prevention. With mounting evidence for a convergence of AD and CVD risk factors, it is also apparent that improving metabolic health more broadly may well pay significant dividends in reducing the burden of these diseases in the future.

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