ALZHEIMER DISEASE

Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease

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Abstract | Alzheimer disease (AD) is a major cause of age-related dementia. We do not fully understand AD aetiology and pathogenesis, but oxidative damage is a key component. The brain mostly uses glucose for energy, but in AD and amnestic mild cognitive impairment glucose metabolism is dramatically decreased, probably owing, at least in part, to oxidative damage to enzymes involved in glycolysis, the tricarboxylic acid cycle and ATP biosynthesis. Consequently, ATP-requiring processes for cognitive function are impaired, and synaptic dysfunction and neuronal death result, with ensuing thinning of key brain areas. We summarize current research on the interplay and sequence of these processes and suggest potential pharmacological interventions to retard AD progression.

Higher executive functioning
Cognitive processes that include planning, reasoning and problem solving that in humans largely involve the prefrontal cortex, with connections to other brain areas.

Reactive oxygen species
(ROS). Oxygen-containing species that contain unpaired electrons (which makes them free radicals) or from which free radicals are easily derived.

Reactive nitrogen species
(RNS). Nitrogen-containing species that are free radicals or moieties from which free radicals are easily derived.

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Alzheimer disease (AD) is characterized by an accumulation of senile plaques (SPs; composed mostly of fibrillary amyloid-β (Aβ) peptide and dystrophic neurites) and neurofibrillary tangles (NFTs; composed of hyperphosphorylated tau protein) in the brain, leading to dysfunction and loss of synapses and eventual neuronal death[1,2]. Clinically, AD is characterized by several features, notably a progressive cognitive decline involving loss of memory and higher executive functioning[1]. Arguably, the earliest stage of AD is preclinical AD (PCAD), in which persons have normal cognitive status but upon death and autopsy their brains display evidence of substantial AD neuropathology. Amnestic mild cognitive impairment (aMCI) is a progressive condition in which there is some degree of memory loss and is widely thought to be a prodromal early stage of AD in which AD neuropathology is present, albeit to a lesser degree. In contrast to patients with AD, however, aMCI individuals can perform activities of daily living. It has been estimated that approximately 15% of people with aMCI progress to AD annually[3].

AD pathology occurs well before (up to two decades) the onset of clinical symptoms[2–4]. It follows that therapy that begins when symptoms appear may be too late to be effective, and understanding key molecular processes in the progression of AD is needed to facilitate earlier diagnosis and to develop new interventions to slow or stop its progression.

One important process that becomes dysfunctional in AD and aMCI is the metabolism of glucose[5,6]. Glucose is normally the major energy source for the brain and is metabolized to ATP via glycolysis, the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC), as shown in FIG. 1. Glucose enters the brain from the vasculature through highly efficient glucose transporters and requires insulin for optimal cellular utilization[7]. In AD and aMCI, however, brain insulin resistance is present[6,7]. Indeed, type 2 diabetes (T2DM), a key component of which is insulin resistance, is a substantial risk factor for developing AD[7]. Given the very large increase in T2DM development worldwide, combined with ageing populations, AD is a major and growing problem.

This Review summarizes the role of oxidative damage in aMCI and AD, how it affects glucose metabolism and how it is a key mechanism behind insulin resistance. We review the reasons for the failures of certain therapeutic approaches in AD and suggest possible new approaches.

Oxidative damage is relevant to AD

Oxidative damage is the damage that is done to biomolecules during oxidative stress (for a detailed review and discussion, see REF.[8]). Oxidative stress is a serious imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and antioxidant defences[8], and has been shown in a wide range of studies to contribute significantly to the pathogenesis and progression of AD[2,8–19]. Diabetes also leads to oxidative stress (REF.[8], also see later discussion), which may make a contribution to its propensity to favour AD development[9]. The term 'reactive' is variable: some ROS and RNS are highly reactive (for example, $OH^{\bullet}$) whereas other are much more selective in their reactions (for example, H_2O_2, $NO^{\bullet}$, $O_2^{\bullet-}$). TABLE 1 lists several biologically important ROS and RNS.

When certain ROS or RNS react with biomolecules, oxidative or nitrosative damage occurs, which can be detected by measuring specific products that result from such damage ('biomarkers of oxidative or nitrosative damage')[8]. Some of the most commonly used biomarkers of oxidative damage to lipids, proteins and nucleic acids are listed in TABLE 2.

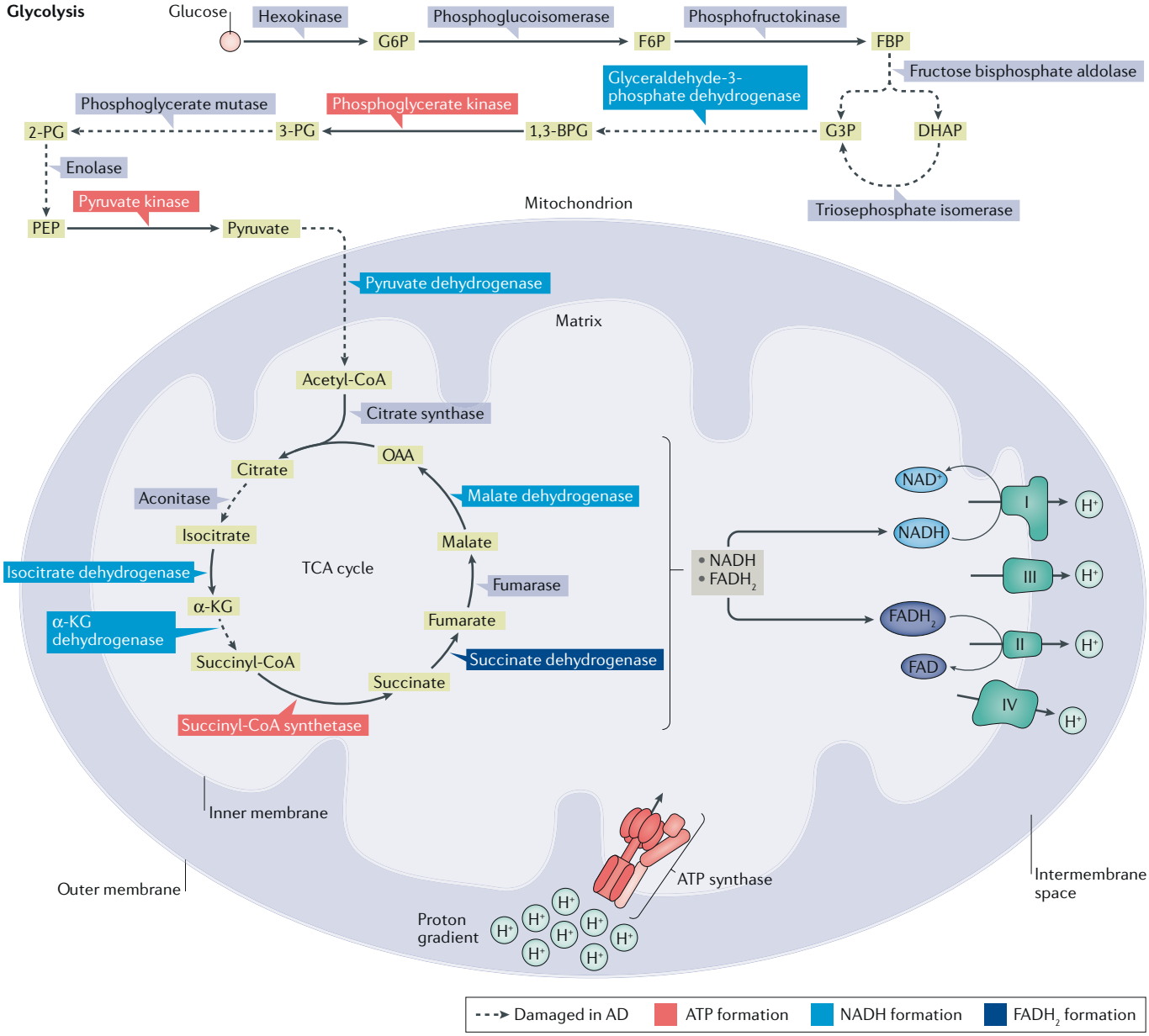


Fig. 1 | **Schematic diagrams of the biochemistry of glucose catabolism and ATP synthesis and their oxidative dysfunction in AD and aMCI brains.** Glycolysis, the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC), the latter localized on the inner mitochondrial membrane, work together to catabolize glucose and drive ATP synthesis via the ATP synthase complex. Complexes I–IV of the ETC are shown. Also shown is ATP synthase, the α-chain of which is oxidatively modified in brains of subjects with Alzheimer disease (AD). Briefly, this figure shows that glucose is converted to pyruvate in glycolysis. Pyruvate is converted to acetyl-CoA, which enters the TCA cycle, and the resulting reducing equivalents (NADH and $FADH_2$) from glycolysis and the TCA cycle enter the mitochondrial ETC. The inner mitochondrial membrane is impermeable to NADH; therefore, the malate–aspartate shuttle leads to NADH synthesis in the matrix via NADH in the cytosol to reduce oxygen to water, leading to production of a mitochondrial proton gradient in the intermembrane space that drives ATP synthesis. Reactions catalysed by specific enzymes or enzyme complexes identified by redox proteomics or other techniques to be oxidatively damaged (and likely thereby dysfunctional) in AD brain (and most also in amnestic mild cognitive impairment (aMCI) brains)[12,22,24–26,34,35,115] are indicated as dashed lines in the figure. 1,3-BPG, 1,3-bisphosphoglycerate; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; α-KG, α-ketoglutarate; DHAP, dihydroxyacetone phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; G3P, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; OAA, oxaloacetate; PEP, phosphoenolpyruvate.

In the brains of individuals with PCAD and AD, levels of oxidative damage to a wide range of molecules are increased[8–19]. For example, levels of protein carbonyls (PCs) are elevated in AD in brain regions that are rich in Aβ-peptide-containing SPs (TABLE 2) but are at normal levels in brain regions devoid of Aβ-rich plaques[19]. Even in patients with aMCI, oxidative damage is already significantly increased: PCs are significantly elevated in

Table 1 | **Some biologically important ROS and RNS**

	ROS	RNS
Radical	• Superoxide, $O_2^{\bullet -}$ • Hydroxyl, $OH^{\bullet}$ • Peroxyl, $RO_2^{\bullet}$ • Alkoxyl, $RO^{\bullet}$	• Nitric oxide, $NO^{\bullet}$ • Nitrogen dioxide, $NO_2^{\bullet}$ • Nitrate, $NO_3^{\bullet}$
Non-radical	• Hydrogen peroxide, H_2O_2 • Hypochlorous acid, HOCl • Organic peroxides, ROOH • Peroxynitrite, $ONOO^-$ • Peroxynitrous acid, ONOOH	• Nitrous acid, HNO_2 • Nitrosyl cation, NO^+ • Nitrosyl anion, NO^- • Peroxynitrite, $ONOO^-$ • Peroxynitrous acid, ONOOH

Note that $NO^{\bullet}$, $ONOO^-$ and ONOOH are classified as both reactive nitrogen species (RNS) and reactive oxygen species (ROS). For a detailed discussion, see REF.[8], from which this table is adapted.

aMCI brains or cerebrospinal fluid (CSF)[8,14,20]. Increased lipid peroxidation (a term explained in TABLE 2) in AD and aMCI brains or CSF and in PCAD hippocampi is further evidenced by rises in the levels of protein-conjugated 4-hydroxy-2-nonenal (HNE), F_2-isoprostanes and F_4-isoprostanes[8,11–13,15,16,19–23]. Elevated levels of 3-nitrotyrosine (3-NT), suggestive of damage by peroxynitrite (TABLE 2), are also observed in AD[18,24] and aMCI[25]. 8-Hydroxy-deoxyguanosine (8-OHdG), a biomarker of oxidative damage to DNA (TABLE 2), is also elevated in AD (in both nuclear and mitochondrial DNA)[26–28], as is oxidative damage to RNA[29,30]. For example, neuritic plaques (rich in fibrillar Aβ42 and Aβ40) and NFTs contain oxidized, glycated and nitrated proteins. Consequences of this increased oxidative and nitrosative damage are likely to include glucose dysmetabolism (see section on oxidative damage below) and loss of ion gradients with resulting impaired action potentials and Ca^{2+} dyshomeostasis. The latter is because oxidative stress is well known to raise intracellular free Ca^{2+} levels, from which several deleterious consequences can follow[8]. Moreover, oxidative DNA damage can interfere with gene transcription and affect promoter function, which can lead to impaired transcription of essential genes and to mutations. Oxidative RNA damage can impair protein translation, and the damaged RNA can be prematurely degraded, further impairing the synthesis of essential proteins (TABLE 2).

Learning and memory deficits, decreased higher executive function and diminished reasoning ability characterize patients with AD, whereas memory deficits are a hallmark of aMCI. In both conditions, these altered functions largely originate from synaptic dysfunction involving altered synaptic proteomes[31,32]. Aβ42 oligomers contribute to this synaptic dysfunction, impairing learning and memory[31]. Aβ42 oligomers also cause oxidative damage to synaptic membranes[12,16], and there seems to be an intimate relationship between this oligomer-induced oxidative damage and synaptic dysfunction. Indeed, the first pathological insults to neurons in AD and aMCI occur at presynaptic and postsynaptic membranes[1,3]. Interestingly, large oligomers of Aβ42, which would have difficulty solubilizing in the neuronal lipid bilayer, seem relatively non-toxic, whereas small oligomers of Aβ42 (for example, dimers or trimers that easily enter lipid bilayers) appear highly toxic to synapses[33]. These considerations support the notion that lipid peroxidation, and perhaps other forms of oxidative damage in synaptic membranes, account for the loss of long-term potentiation and other synaptic functions involved in learning and memory[12,15,16,20–22,34,35].

Dysfunctional glucose metabolism in AD

The brain is an energy-demanding organ and relies heavily on efficient ATP production via glycolysis, the TCA cycle and oxidative phosphorylation[7] (FIG. 1). However, glucose metabolism in AD and aMCI brains is significantly impaired[5–7,9,36]. What causes this loss of glucose utilization?

Contribution of oxidative damage. Research from our laboratories[11,12,34] and many others[2,4,8,10] has shown that inefficient glucose utilization (and thus impaired ATP production) and oxidative damage are intimately related. A major contributor to inefficient glucose utilization may well be oxidative modification, which often leads to decreased activity of the enzymes involved in glucose metabolism (FIG. 1).

The techniques of redox proteomics[34] allowed specific oxidatively or nitrosatively modified proteins (that is, PC-modified, protein-bound HNE-modified and/or 3-NT-modified proteins) to be identified in the brain from subjects with late-stage AD, aMCI and PCAD[20,24,34,35]. For example, redox proteomics of AD brain tissue revealed that in affected brain areas, oxidative modification of the glycolytic enzymes aldolase, triosephosphate isomerase (TPI), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate mutase 1 (PGAM1) and α-enolase occurs[12,34]. In addition, oxidative modifications to aconitase (a key iron–sulfur-containing enzyme in the TCA cycle), creatine kinase (an enzyme that helps neurons to maintain ATP levels) and ATP synthase in brain mitochondria help to explain decreased glucose metabolism and consequent decreased ATP production in the brains of people with aMCI and AD[12,25,34]. Oxidative damage to mitochondrial DNA[27,28] might also contribute to impaired energy production, and there has been a suggestion that defects in sirtuin 3 (SIRT3) contribute to oxidative damage in AD mitochondria[37]. Indeed, mitochondrial dysfunction and insulin resistance are intimately related[7,36].

The consequences of this decreased ATP production in AD and aMCI are profound. For example, decreased ATP will diminish the neuron's ability to maintain ionic gradients, hindering production and propagation of action potentials and therefore neurotransmission. Moreover, loss of ion gradients can allow extracellular Ca^{2+} to enter, which can further raise intracellular free Ca^{2+} levels, stimulating Ca^{2+}-dependent endonuclease, phospholipase and proteinase activities[8], contributing to synaptic dysfunction and eventual neuronal death. Excess Ca^{2+} can saturate the ability of the endoplasmic reticulum (ER) and mitochondria to buffer and cycle Ca^{2+}, causing swelling of the latter with consequent opening of the mitochondrial permeability transition pore, leading to release of cytochrome *c* and apoptosis-inducing factor 1, provoking neuronal apoptotic death[38]. Excess intraneuronal free Ca^{2+} can

Redox proteomics
A method for identification of oxidatively modified proteins that most often involves protein separation and digestion, mass spectrometric utilization to sequence the amino acids of the resulting peptides and protein identification and informatics.

Table 2 | **Some of the major biomarkers of oxidative damage in brain**

Target	Products measured	Comments	Refs
Proteins	3-Nitrotyrosine	Mostly produced when a superoxide radical ($O_2^{\bullet-}$) reacts with nitric oxide ($NO^{\bullet}$) to give peroxynitrite ($ONOO^-$), which leads to protein nitration on Tyr (and other) residues by complex mechanisms	8,18
	• Protein carbonyls • Methionine sulfoxide — product of attack by ROS and/or RNS on methionine residues • Highly reactive aldehydes, for example, 4-hydroxy-2-nonenal (HNE), hexenal (HHE) and 2-propene-1-al (acrolein). These latter molecules can form covalent adducts by Michael addition reactions with Cys, Lys and His residues on proteins. Such Michael adducts alter the conformation and function of proteins and can be detected by immunochemical or mass spectrometric methods	There are four principal mechanisms that generate protein carbonyls: • Free radical scission of the peptide chain, leading to aldehyde-containing fragments • Oxidation of amino acid side chains (e.g. formation of oxo-histidine) • Covalent attachment to proteins of aldehydic lipid peroxidation products (discussed below) • Formation of advanced glycation end products	8,14,15,118
Lipids	• Lipid peroxides • Cyclic peroxides	Lipid peroxidation usually results from abstraction of labile allylic H atoms from unsaturated fatty acid side chains of phospholipids by ROS and/or RNS. This abstraction generates carbon-centred radicals ($C^{\bullet}$). Subsequent chain reaction steps involve: • Binding of O_2 (which has two unpaired electrons and is lipid soluble) to the $C^{\bullet}$ by very rapid radical–radical recombination to form a peroxyl free radical ($LO_2^{\bullet}$) • Reaction of this peroxyl radical with another allylic H atom on an adjacent acyl chain (or sometimes elsewhere on the same acyl chain) of phospholipids to form a lipid hydroperoxide, LOOH (or sometimes a cyclic peroxide), thereby reforming a $C^{\bullet}$ free radical. This chain reaction will repeat as long as labile H atoms are available	8,11,13–16,119
	• F_2-isoprostanes • F_3-isoprostanes • F_4-isoprostanes	Isoprostanes are a family of lipid peroxides mostly derived from arachidonic (F_2), eicosapentaenoic (F_3) or docosahexaenoic (F_4) acids. They are often regarded as the most reliable 'gold-standard' biomarkers of lipid peroxidation (especially F_2 and F_4)	119
DNA	• 8-Hydroxy-deoxyguanosine (8OHdG) • A range of other base oxidation products	8OHdG is a mutagenic product and the most commonly measured biomarker of oxidative DNA damage. It is generated by attack of several ROS and/or RNS on guanine residues in DNA. However, some highly reactive ROS and/or RNS, such as $OH^{\bullet}$ and ONOOH, generate a range of other products in addition	8,120
RNA	8-Hydroxyguanine (8OHG)	8OHG is the most commonly measured biomarker of oxidative RNA damage and is generated by attack of several ROS and/or RNS on guanine residues in RNA. RNA oxidation has a range of deleterious consequences	8,121–123

Increased levels of biomarkers of oxidative and nitrosative damage can be due to increased reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) production, decreased clearance of oxidatively damaged molecules or most likely both. Each of these processes plays a role in Alzheimer disease and amnestic mild cognitive impairment.

also cause loss of fidelity of microtubule assembly[39], with consequent decreased anterograde and retrograde transport of mitochondria and neurotransmitter vesicles, starving presynaptic terminals of energy and decreasing neurotransmission, which, in turn, leads to synaptic dysfunction, neuronal death and ultimately cognitive dysfunction.

mTOR activation and AD. Brain insulin resistance is common in AD[7,36,40]. One of the mechanisms by which insulin resistance can develop is by activation of the mechanistic target of rapamycin (sometimes called the mammalian target of rapamycin), usually abbreviated mTOR. mTOR is a highly integrated complex of many proteins[40,41] and exists in two functionally distinct forms, mTORC1 and mTORC2. Activated mTORC1 is intimately involved in regulation of protein synthesis, autophagy, mitochondrial function, lipogenesis, ketogenesis and insulin signalling and is crucially linked to glucose metabolism, where it becomes activated by growth factors, amino acids and high cellular energy status[40,41] (FIG. 2).

Inhibition of autophagy following activation of mTOR in AD (FIG. 2) causes accumulation of aggregated, misfolded proteins and damaged organelles, particularly mitochondria, which can lead to inhibition of normal cellular processes. This important mediator of neuronal death is present in the early stages of AD, and evidence of impaired autophagy is also found in aMCI brains and PCAD brains[40–42]. Insulin resistance is another detrimental consequence of mTOR activation in aMCI and AD brains[40] (FIG. 2). These mTOR-mediated events may help explain the observation that T2DM is an important risk factor for the development of AD[7,40]. In addition to

Autophagy
One of the components of the proteostasis network; involves formation of a double membrane (autophagosome) that surrounds the aggregated, damaged protein or organelle and transport of the autophagosome to and fusion with a lysosome, exposing the contents of the autophagosome to proteolysis and degradation.

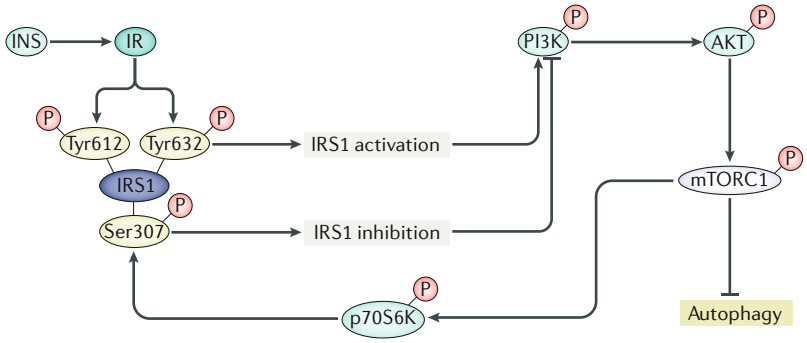


Fig. 2 | **Schematic representation of biochemical events associated with insulin binding to its receptor, leading to activation of mTORC1 with subsequent inhibition of autophagy and development of insulin resistance.** After insulin (INS) binds to the insulin receptor (IR) on neuronal membranes, the IR dimerizes and autophosphorylation on tyrosine residues occurs. Insulin receptor substrate 1 (IRS1) recognizes IR phosphotyrosine residues and binds to the IR, which in turn leads to phosphorylation of IRS1 on Tyr612 and Tyr632 with resultant activation of IRS1. Activated IRS1 leads to phosphorylation and activation of two pathways for the insulin signalling cascade, one of which is the PI3K–AKT pathway. Phosphorylated PI3K leads to phosphorylation and activation of AKT, which leads to phosphorylation of Ser2448 of the mechanistic target of rapamycin complex 1 (mTORC1), the latter becoming activated as a kinase[7,40,41,61]. Activated mTORC1 kinase has several key downstream effects (two of which are shown in the figure) that impair neuronal survival (and are thus relevant to Alzheimer disease), including inhibition of autophagy and phosphorylation of the protein p70S6K, which then becomes a kinase, one of the substrates of which is Ser307 of IRS1. Once phosphorylated on Ser307, IRS1 function ceases, leading to and becoming a marker for insulin resistance[34,40,61].

mTOR activation, insulin resistance can also result in neuronal glucose deficiency, with consequent decreased glucose metabolism, impaired ATP production and dysfunction and/or death[7,41].

Glycation and brain oxidative damage. When reducing sugars react with the side chain amino groups of lysine residues on proteins, several processes occur that eventually generate advanced glycation end products (AGEs). AGE formation involves not only direct reaction of these residues with the sugars but also oxidative damage to the proteins: the combination of these reactions is often referred to as glycoxidation (reviewed in REFS[8,43]). AGEs are ligands for receptors for AGEs (RAGEs)[43–46]. Glycoxidation is highly relevant to AD, in part because Aβ extracellular fibrillar aggregates have characteristics of AGEs and bind to RAGE in neurons and brain endothelial cells. AGE and Aβ binding to RAGEs leads to further oxidative stress that contributes to neuronal death and vascular dementia in AD[44–47]. Glycation may slow the conversion of Aβ to fibrils, keeping them longer in the toxic oligomeric forms[47]. Vascular dysfunction worsens cognitive defects in AD[48].

Proteostasis abnormalities in AD. The proteostasis network consists of autophagy (all types), the ubiquitin–proteasome system (UPS) and the unfolded-protein response (UPR) in the ER[42,49–53] (FIG. 3). One might predict that as a consequence of decreased autophagy in AD (see above), the UPS would become hyperactivated to compensate for decreased removal of damaged proteins. In fact, proteasome activity seems to be decreased in AD[49–51]. In addition, a crucial part of the UPS is oxidatively damaged and dysfunctional in both aMCI and AD brain — namely, ubiquitin carboxy-terminal hydrolase L1 (UCHL1)[34,54–56]. UCHL1 removes (one residue at a time from the carboxyl terminal) the polymeric ubiquitin chains that have been added to the damaged proteins by ubiquitin ligases so that the protein can enter the 19S cap of the 26S proteasome. In aMCI and AD brains, the failure of oxidatively damaged UCHL1 to perform its function leads to accumulation of ubiquitinylated proteins, decreased proteasome function and subsequent accumulation of damaged, aggregated proteins[54]. This UCHL1 dysfunction synergizes with dysfunctional autophagy, ER stress (described below) and other impairments of the proteostasis network (FIG. 3) to contribute to the cellular detritus in neurons, hastening their demise. In the presence of excessive oxidative stress, the two 19S caps can be separated from the 26S proteasome, leaving the 20 S proteasome capable of degradation of certain oxidatively damaged proteins without the involvement of ubiquitin[49]. One implication of the ability of the 20 S proteasome to degrade oxidatively modified proteins independent of the 19S components of the 26S proteasome is that the involvement of UCHL1 is not needed, and, as noted above, this critical enzyme is oxidatively dysfunctional in AD. This could be regarded as an attempt to rescue dysfunctional proteostasis. Unfortunately, degradation of oxidatively modified proteins is still compromised in AD brain as the 20 S proteasome enzymatic activities are dysfunctional[50,51] (including inhibition by Aβ), resulting in accumulation of oxidatively modified, often misfolded and dysfunctional proteins.

In response to abnormal elevated levels of misfolded proteins and/or free Ca^{2+} in the ER lumen, the UPR is engaged[52,53,57]. This UPR engagement consists of activation of one or more of three stress transducers that lead to rapid responses apparently evolved to repair the defective mechanisms that caused the cellular dysfunction: protein kinase R-like ER kinase (PERK; encoded by *EIF2AK3*), inositol-requiring enzyme 1α (IRE1) and activating transcription factor 6α and 6β (ATF6). Each stress sensor induces one or more downstream response mechanisms that are designed to lower ER stress (FIG. 3). Normally, these three stress sensors are inactive owing to interaction with binding immunoglobulin protein (BiP; also known as glucose regulated protein 78 (GRP78)), which, when removed, leads to activation of the stress response. Addition of oligomeric Aβ to neurons leads to large elevations in Ca^{2+} with consequent oxidative stress and ER-stress-dependent neuronal death. Inhibition of autophagy, oxidative stress and mitochondrial function loss also activate the UPR and, as noted above, all of these alterations are observed in AD[34,40–42,52,53,57] (FIG. 3). For PERK, receptor tyrosine kinase (RTK) activity phosphorylates eIF2α, which leads to decreased protein translation and causes elevated rates of translation of normally poorly translated mRNAs, among which is ATF4, which, in turn, leads to decreased redox homeostasis and elevated apoptosis. The ATF6-transduced ER stress response involves inducing transport of the ATF6 precursor protein to the Golgi apparatus, where a shorter form of ATF6 is produced. This shorter form

Proteostasis
Sometimes called protein quality control, proteostasis is a term encompassing three different cellular processes (the ubiquitin–proteasome system, autophagy and the endoplasmic-reticulum-resident unfolded-protein response) used to degrade aggregated, damaged proteins or sometimes cellular organelles.

then translocates to the nucleus, where expression of X-box binding protein 1 (XBP1) occurs. In the case of IRE1, transduced ER stress results in dimerization and formation of a transmembrane kinase, RTK, which, in turn, results in RNA degradation by regulated IRE1-dependent decay (RIDD); XBP1-mediated alternative mRNA splicing that leads to ER-associated degradation (ERAD) and increased lipid metabolism, which can lead to elevated levels of the lipid peroxidation product, HNE; and tumour necrosis factor receptor-associated factor 2 (TRAF2)-mediated inflammatory or pro-apoptotic gene induction, including nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK). In AD brain, markers of ER stress are elevated and correlate with progression of this disorder[52,53,57].

Thus, three key components of the proteostasis network are dysfunctional in aMCI and AD brains. This dysfunction contributes to neuronal damage and death by several mechanisms, including ER stress and the accumulation of oxidatively damaged proteins, which in the healthy brain are usually degraded by the UPS and autophagy to keep their levels low[8,49,52,54].

In summary, oxidative and/or nitrosative damage to multiple proteins, including synaptic proteins, is very likely to contribute to the memory problems and other cognitive deficits, reduced ATP availability in neurons and decreased clearance of abnormal proteins[34], creating a 'perfect storm' (or sTORm perhaps) of detrimental cognitive effects in AD[9,34,35,40,41].

Down syndrome and AD

Further evidence for a key role of oxidative damage in AD comes from studies of Down syndrome (DS). The varied phenotypes of DS result from full or partial triplication of chromosome 21, the most common human chromosomal disorder[58]. There are several genes associated with AD and oxidative stress on chromosome 21, including those encoding amyloid precursor protein (APP), from which Aβ arises, and β-secretase 2, one of the proteinases that act on APP to form Aβ. One striking characteristic of people with DS is that at approximately 40–50 years of age, AD-like dementia often appears[58,59]. It is likely that both a dose effect of triplication of *APP* and gene–environment interactions play a role[58,59]. Other proteins encoded on chromosome 21 may be involved[58], such as Cu/Zn-superoxide dismutase (which is an important antioxidant enzyme normally but can be deleterious if excess is present[8]) and BACH1 (transcriptional inhibitor of haem oxygenase 1 (HO-1)). HO-1 is an important cellular antioxidant system that degrades free haem (a pro-oxidant)[8], and interference with its action can contribute to oxidative stress[8,60,61].

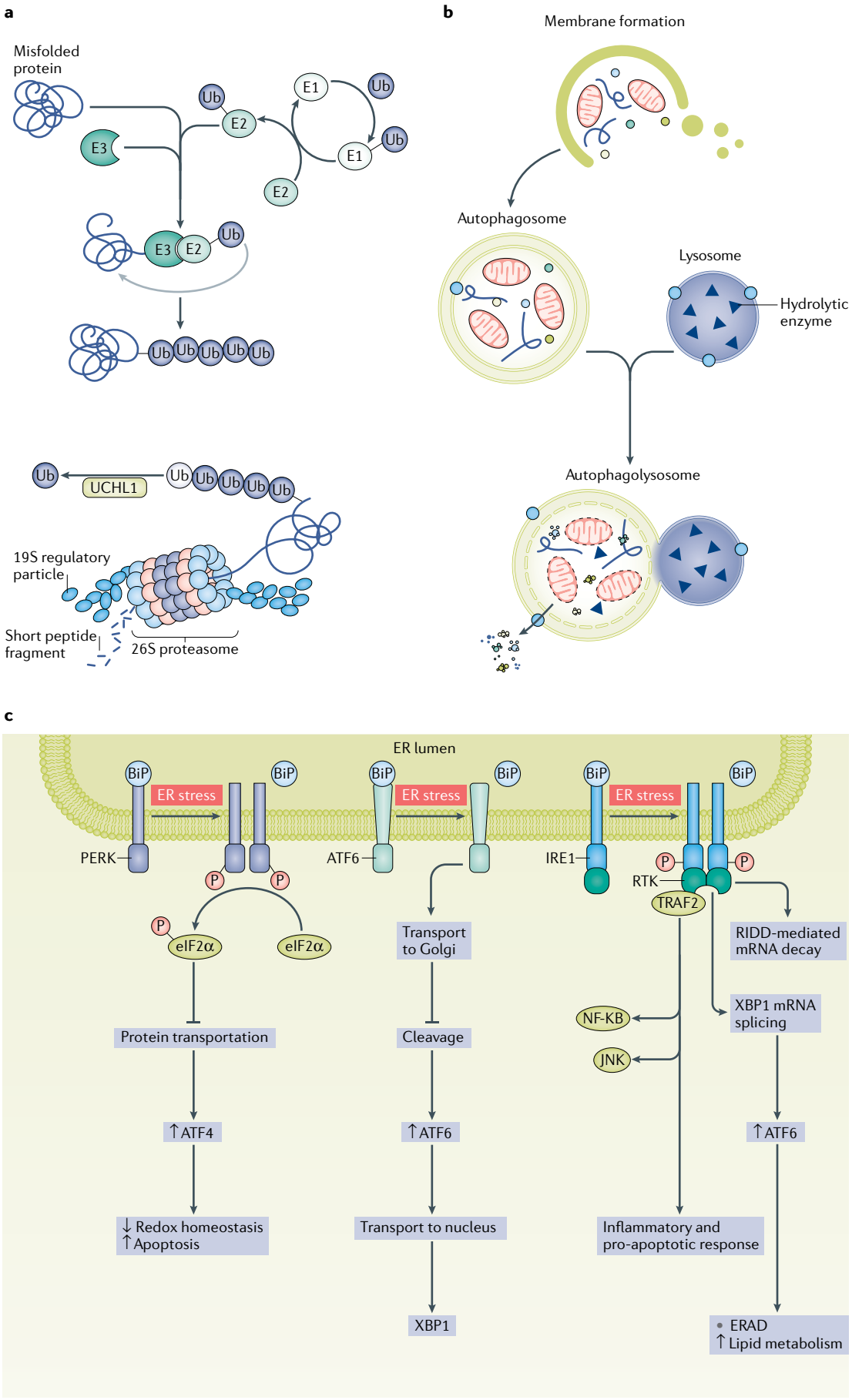
Increased oxidative damage occurs early in DS development, as evidenced by the fact that neurons obtained from aborted fetuses with DS show evidence of increased oxidative damage[62], as does amniotic fluid from mothers carrying fetuses with DS[63]. These observations are consistent with the notion that oxidative damage contributes substantially to the pathogenesis and progression of DS[41,63]. Redox proteomics has identified several oxidatively damaged proteins (including ceruloplasmin, transferrin, retinol-binding protein 4, apolipoprotein A-I (APOAI), complement C9 and collagen α1(V) chain) in amniotic fluid that correlate with the various phenotypes presented in individuals with DS, suggesting that oxidative stress contributes to these phenotypes[63]. Other biomarkers of increased oxidative damage in the brains of persons with DS and persons with DS with AD neuropathology (DS with AD) include elevated levels of HNE and PC[15,41]; the increases are greater in people with DS with AD[61,64,65]. Moreover, redox proteomics identified brain proteins oxidatively modified by PC and HNE that are similar to those in AD[41,64]. These oxidatively modified, and likely dysfunctional, proteins include ones associated with altered glucose metabolism, mTOR signalling (including inhibited autophagy and increased insulin resistance) and the proteostasis network[41,60,61,64,65]. All of these events will impair brain function and development in DS.

Proteins encoded on chromosome 21 and excess levels of Aβ and hyperphosphorylated tau protein[41] constitute a vicious cycle for neuronal damage in DS. Elevated oxidative stress and mTOR activation with consequent inhibited autophagy that results in elevated levels of neuronal detritus are observed, and AD-like SPs form. Among the non-degraded moieties is Aβ itself, the accumulation of which leads to more oxidative damage, RAGE activation and activation of mTOR, continuing the vicious cycle. Hyperphosphorylated tau leads to destabilization of microtubules, affecting mitochondrial trafficking and thus leading to presynaptic energy deficits. Consequent loss of ion gradients in presynaptic membranes that is secondary to loss of mitochondrial anterograde transport down axons, with consequent damaging Ca^{2+}-related changes (discussed earlier), occurs[12,24,34,41]. Tau hyperphosphorylation also facilitates deposition of NFTs similar to those in AD brain. Furthermore, activated mTOR-mediated phosphorylation of insulin receptor substrate1 (IRS1, via phosphorylated p70S6K; FIG. 2) leads to insulin resistance in DS, contributing to glucose hypometabolism. It is conceivable that mTOR-mediated alteration of insulin signalling due to IRS1 inhibition, coupled with glucose hypometabolism, could lead to elevated tau phosphorylation, causing NFT formation and neuronal death[7,41,66].

To summarize, the genetic abnormalities underlying DS drive mechanisms similar to those in AD, providing insights not only into DS but also into aMCI and AD and suggesting novel therapeutic targets and approaches[67]. Indeed, ongoing investigations of the temporal changes in damaged brain proteins associated with glucose metabolism, the proteostasis network and glutamate metabolism (the latter changes related to the functioning of the TCA cycle) are likely to provide insights into the molecular bases for conversion of DS into DS with AD.

Early detection is important

As mentioned earlier, brain pathology in patients who will eventually develop AD probably begins at least two decades before clinical symptoms appear[2,4]. Therefore, identifying who is at risk of developing AD and early detection of the disease are essential to allow potential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Misfolded protein
E3
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19S regulatory particle
Short peptide fragment
26S proteasome
b
Membrane formation
Autophagosome
Lysosome
Hydrolytic enzyme
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ER stress
PERK
ATF6
IRE1
RTK
TRAF2
P
eIF2α
Transport to Golgi
RIDD-mediated mRNA decay
XBP1 mRNA splicing
NF-KB
JNK
Protein transportation
Cleavage
↑ATF4
↑ATF6
↑ATF6
↓ Redox homeostasis
↑ Apoptosis
Transport to nucleus
Inflammatory and pro-apoptotic response
XBP1
• ERAD
↑ Lipid metabolism

◀ Fig. 3 | **Schematic drawings of the three components of the proteostasis network in brain cells. a** | The ubiquitin–proteasome system. Damaged proteins are polyubiquitinylated by the ubiquitin ligase enzymes E1, E2 and E3. E1 requires ATP for its function. An initial ubiquitin molecule is bound to the damaged protein or organelle by this process and is repeated to form a polyubiquitinylated chain. Polyubiquitinylated damaged proteins are destined for degradation by the 26S proteasome, but prior to entering the 19S cap, these proteins must be de-ubiquitinylated, one ubiquitin residue at a time, by the enzyme, ubiquitin carboxy-terminal hydrolase L1 (UCHL1). The de-ubiquitinylated, damaged protein is degraded by the proteinases in the 20S portion of the 26S proteasome, and small peptides are ejected by the bottom 19S portion of the 26S proteasome to become degraded by soluble peptidases into individual amino acids for reuse[54–56]. **b** | Autophagic degradation of aggregated proteins or organelles. The process starts with the formation of a double membrane enveloping the aggregated, damaged protein or organelle to form an autophagosome. This is transported to the lysosome, where membrane fusion leads to formation of the autophagolysosome. Endocytosis of the contents of the autophagosome into the acidic interior of the lysosome leads to their proteolytic degradation, with peptides, amino acids and other biomolecules being ejected from the autophagolysosome for reuse[42]. **c** | The unfolded-protein response (UPR) associated with endoplasmic reticulum (ER) stress. Following an elevation in the levels of misfolded proteins and/or Ca^{2+} in the ER lumen, the UPR is usually engaged. This engagement of the UPR consists of activation of one or more of three stress transducers: protein kinase R-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1) and activating transcription factor 6α and 6β (ATF6). Activation of each stress sensor is accompanied by removal from the stress sensor of binding immunoglobulin protein (BiP), which when bound inactivates each of the three stress transducers. Each activated stress sensor induces one or more downstream response mechanisms. In the figure, up arrows denote an increased process or level whereas down arrows denote a decreased process or level. For PERK, receptor tyrosine kinase (RTK) activity phosphorylates eIF2α, which leads to decreased protein translation, which causes elevated rates of translation of normally poorly translated mRNAs, among which is ATF4. In turn, this leads to decreased redox homeostasis and elevated apoptosis. ATF6 transduces ER stress by inducing transport of the ATF6 precursor protein to the Golgi apparatus, where the shorter ATF6 is produced. The latter translocates to the nucleus, leading to expression of X-box binding protein 1 (XBP1). In the case of IRE1, transduced ER stress results in dimerization and formation of a transmembrane kinase, RTK, which in turn results in RNA degradation by regulated IRE1-dependent decay (RIDD); XBP1-mediated alternative mRNA splicing that leads to ER-associated degradation (ERAD) and increased lipid metabolism (from which elevated levels of the lipid peroxidation product, 4-hydroxy-2-nonenal (HNE) can arise); and tumour necrosis factor receptor-associated factor 2 (TRAF2)-mediated inflammatory or pro-apoptotic gene induction, particularly those of nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK). The ER-resident component of the proteostasis network, particularly the UPR, is impaired in amnestic mild cognitive impairment and Alzheimer disease (AD) brains owing to accumulation of abnormal proteins and alterations in Ca^{2+} homeostasis[52–54,57]. In AD brains, markers of ER stress are elevated and correlate with progression of the disease[52–54,57]. Adapted with permission from REF.[57], Wiley-VCH.

disease-modifying treatments to be administered before substantial neuronal dysfunction and death have occurred. Simple blood tests to detect early development of AD pathology would be the 'Holy Grail', and there has been some progress towards this goal. A recent study[68] measured plasma Aβ biomarkers by immunoprecipitation coupled with mass spectrometric analysis. Plasma from hundreds of well-characterized controls and from patients with aMCI and AD from Japan and Australia was examined. The results demonstrated, with very high confidence (over 94% of samples tested), that Aβ biomarkers in plasma can correctly discriminate between control, aMCI and AD subjects. This study raises hopes that this far-less-invasive approach (compared with lumbar puncture to obtain CSF) will allow physicians and scientists to both treat patients earlier in the progression of this disorder than is currently possible and gain insights into the molecular processes involved in the aetiology and progression of AD. Exosomal biomarkers might be another promising approach in DS with AD[69].

CSF biomarkers have also been proposed but not yet validated; however, these are less desirable than plasma biomarkers because extracting CSF is more invasive[2,70]. The use of biomarkers detected through retinal imaging has also been proposed[2,71,72]. Another approach underway to address this need for predictive biomarkers in AD involves the Dominantly Inherited Alzheimer Network (DIAN)[73]: persons carrying one of several dominantly inherited genes that cause familial AD agreed to provide fluids and tissue and undergo neuroimaging to detect AD pathology. The combined data allowed researchers to gain insights into factors and biomarkers that might predict onset of AD pathology and clinical symptoms. The results of studies using the DIAN should be extremely helpful in AD treatment and AD research. Indeed, a recent neuroimaging study using the DIAN shows that preferential degradation of cognitive networks differentiates AD from normal ageing[74]. Other valuable networks contributing to our understanding of the above events have been the AIBL (Australian Imaging Biomarkers and Lifestyle) study[2] and the ADNI (Alzheimer's Disease Neuroimaging Initiative)[75] as well as cohorts of people with DS[67,69].

Using a different approach, oxidative stress was found to be elevated in mitochondria isolated from peripheral lymphocytes of people with aMCI and AD[76], results that potentially could be part of a putative panel of biomarkers to identify development of aMCI or AD prior to the appearance of symptoms. Given the involvement of oxidative stress in the progression and pathogenesis of AD, it is possible that unless AD therapies (such as antioxidants) are used much earlier in AD than the onset of symptoms, they will have limited effectiveness and may be totally unsuccessful. Therefore, let us move on to consider the value of antioxidants.

Antioxidant interventions in AD

Vitamin E. The key role of oxidative damage in the pathology of aMCI, PCAD and AD suggests that inhibiting oxidative damage should have therapeutic benefit[8,11]. Consistent with this view, many preclinical models of aMCI and AD, such as neuronal cell cultures or transgenic mice, have shown significant protective effects of antioxidant treatment (for example, REFS[2,8,77–79]). Therefore, it is surprising that the results of clinical trials in aMCI and AD involving antioxidant therapies (such as vitamin E) have been largely disappointing[2,8,70,78,80].

The trials that have been conducted using vitamin E are worth examining because they may have lessons relevant to the use of other antioxidants. Following a report of success of high-dose vitamin E in keeping patients with late-stage AD out of long-term care facilities longer than patients on a placebo[81], other studies with vitamin E have been less impressive[70,78,80]. So what went wrong? Although vitamin E administration has been observed to decrease oxidative damage levels in brain in vivo in some studies[82], administering extra vitamin E has not proved particularly effective as an antioxidant in humans in vivo[8]. If administered vitamin E does not significantly decrease oxidative damage in the brain, then it will not

be effective against MCI or AD. One reason is that transport of extra vitamin E into the brain is limited[83].

The most important biological form of vitamin E is *RRR*-α-tocopherol, the major form found in the brain. However, three other tocopherols (β, γ and ξ) are known, with frequent suggestions (although limited evidence) that they may be important antioxidants in vivo[8]. Although α-tocopherol is efficient in trapping lipid peroxyl radicals, γ-tocopherol appears more efficient at scavenging RNS[84]. Because both elevated oxidative and nitrosative stress occur in AD (see above), clinical trials of vitamin E in AD should perhaps have included both of these forms of tocopherol.

Polyphenols. Some so-called antioxidant molecules (for example, polyphenols such as resveratrol or quercetin) are able to enter the brain to a limited extent[85]. In preclinical models, such molecules have shown promise in the treatment of AD[77]. One mechanism by which polyphenols could exert protective properties is by generation of a hormetic response to their use[85–88]. In other words, they generate a mild oxidative stress that the body tries to mitigate by upregulating protective genes. This often leads to increases in the levels of antioxidants such as glutathione and HO-1, mediated by activation of the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2)[8,89].

How does this system work? NRF2 is widely expressed in animal tissues, but most of it is kept in an inactive form, largely in the cytosol, by binding to Kelch-like erythroid cell-derived protein with CNC-homology associated protein 1 (KEAP1), which also promotes its rapid degradation by the proteasome[8,89]. Many xenobiotics induce oxidative stress and are, or can form, electrophiles, agents that react with sites of high electron density on proteins, DNA or lipids (an example being unsaturated aldehydes such as HNE formed during lipid peroxidation; TABLE 2). An increase in oxidative and electrophilic stress can activate protein kinases that phosphorylate cytoplasmic NRF2, causing it to fall away from KEAP1. The NRF2 then migrates into the nucleus and binds to an antioxidant response element to initiate gene transcription. In addition, KEAP1 is rich in cysteine residues, and its direct modification by ROS and/or RNS and electrophiles, including α,β-unsaturated aldehydes, and dietary xenobiotics such as sulforaphane or curcumin can also stop the proteasomal degradation of NRF2 and cause it to accumulate[89]. Indeed, impairment of NRF2 function worsened cognitive defects in a mouse model of AD[90], suggesting the importance of the NRF2 system in AD. In vivo use of polyphenols (especially curcumin) in some mouse models of AD has been successful[77,87], but this has not (yet) been translated to usefulness in human AD[2,70,87,88].

However, one must be prudent when using polyphenols as 'antioxidant' therapeutic agents. If their beneficial effects actually rely on a mild pro-oxidant action that triggers hormesis, too many of these molecules in the brain would likely aggravate oxidative stress rather than ameliorate it. There can be other problems. For example, the polyphenol curcumin, found in turmeric, has been widely used in preclinical studies, several of which suggest that its antioxidant properties, ability to promote Aβ clearance and other actions may be a potential treatment for AD[2,87,88]. However, curcumin is actually a poor antioxidant in vitro[8] and readily breaks down (for example, in cell culture[91]) to a range of biologically active products. Curcumin can bind to many proteins and membranes, sometimes disguising in in vitro assays the effects of potential new drugs that might eventually prove useful for AD or other disorders[89]. The true value of curcumin in AD therapy remains unclear[70]. Brain-penetrant antioxidant and cytoprotective agents without pro-oxidant effects, such as ergothioneine, may be a promising approach[92,93]. Other approaches include the use of different agents, such as triterpenoids, that can activate NRF2 (REF.[94]), although they (and other agents that act via NRF2) should be used with caution because too much NRF2 activation can be deleterious[89,95]. Mitochondrially targeted antioxidants, to reduce mitochondrial oxidative damage in AD[94,96], might also prove useful.

Deuterated lipids. Lipid peroxidation involves abstraction of labile hydrogen atoms from unsaturated fatty acid side chains (TABLE 2). Chains in which labile hydrogen atoms are replaced by deuterium atoms are more resistant to peroxidation. This is because abstraction of deuterium by ROS is harder because the C–D bond is much stronger than the C–H bond. Deuterated lipids have been claimed to be effective in some preclinical models of AD[97], but more research is needed to establish their true value.

Other interventions

Intranasal insulin. Craft and co-workers investigated insulin resistance in AD[7,36] and reported improved cognition in patients with early AD and decreased levels of neurotoxic Aβ42 following a short-term treatment with intranasal insulin[98]. The results differed according to gender and *APOE* genotype[98]. This potentially important intervention to slow or halt the progress of AD requires an understanding of the molecular basis of the improvement. Recently, intranasal treatment of a 3xTg mouse model of AD with insulin showed a significant reduction in brain nitrosative stress, tau phosphorylation and Aβ oligomers coupled with improved cognition, and these effects were dependent on the activity of biliverdin reductase A (BVRA)[99,100]. BVRA, in addition to being a reductase, is a kinase that can phosphorylate IRS1 (REF.[100]). BVRA is nitrosatively modified and dysfunctional in AD and MCI brain[100], and its impairment promotes insulin resistance[101]. Further studies of the molecular processes involved in cognitive improvement in AD and MCI by intranasal insulin treatment likely will be driven by the results of an ongoing multicentre clinical trial using intranasal insulin.

Alternative energy sources? In certain circumstances, the brain can use ketone bodies (acetoacetate and β-hydroxybutyrate) as metabolic fuel[102]. Thus, it has been suggested that a ketogenic diet might have some beneficial effect in aMCI and mild to moderate AD because brain ketone body metabolism seems unchanged in AD[102].

Its value is uncertain as yet[70]. Studies on human *APOE* gene-targeted female replacement mice show that APOE status appears to influence brain glucose and ketone metabolism, with the *APOEε4* allele being the most deleterious for the former[103].

Diet and lifestyle. An additional consideration when considering the effectiveness (or lack of it) of antioxidants is that, although aMCI and AD are strongly associated with oxidative damage, they are highly complex disorders, and processes other than oxidative damage are likely to contribute to their pathogenesis and progression. For example, APP is processed in several ways by proteinases; therefore, in addition to Aβ42 oligomers, the soluble β-secretase fragment and parts of the carboxy-terminal fragments of APP are reportedly neurotoxic[104,105]. Consequently, therapeutic approaches for aMCI and AD probably need to be multifactorial. Towards this end, studies involving aged beagle dogs, which accumulate Aβ42 and Aβ40 brain deposits with an amino acid sequence identical to that of humans, have proved illustrative. The study found that aged beagles (12 years old) placed on a high-antioxidant diet, given environmental enrichment to produce more cognitive stimulation (resulting in more synapse formation) and provided exercise for 3 years (exercise leads to elevation of brain-derived nerve growth factor, among other benefits[106]) had an error rate on behavioural tests, brain Aβ42 levels and brain oxidative damage similar to 4-year-old dogs[107]. If this promising result is translatable to humans, then at a certain age (for example, age 40 years, to account for the pathology of AD being present for at least two decades prior to appearance of clinical symptoms of aMCI or AD[4]), or ideally throughout a lifetime, persons should eat a diet high in antioxidants, develop new intellectual tasks (such as learning a new language, learning to play a new musical instrument and more) and undertake a reasonable degree of exercise. Several studies have indicated that these approaches can yield results[2,108,109]. A recent study is the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER). This study was a 2-year intervention consisting of diet, exercise, cognitive training and management of metabolic and vascular risk factors. Positive effects on cognition were reported[108,110] even in subjects with the *APOEε4* allele[110]. By contrast, single interventions (for example, decreasing vascular risk, as in the preDIVA trial, or administering nutritional supplements) seem in general to be less effective[70,109,111].

Indirect antioxidants. A final consideration of this topic is the potential treatment of aMCI and AD with indirect antioxidants[8] — agents that can decrease oxidative stress but not by direct antioxidant mechanisms. Polyphenols may be one example, acting as mild pro-oxidants (see above). Another example is the intersection of oxidative stress, glucose metabolism and statins, such as atorvastatin, as promising agents to decrease damage in aMCI and AD brains[112].

The enzyme HO-1 oxidizes haem (a pro-oxidant)[8] to produce biliverdin, which is reduced by the BVRA enzyme to bilirubin (a scavenger of ROS and RNS[8,101]). Both HO-1 and BVRA are oxidatively damaged in aMCI and AD brains[100]. Moreover, oxidative modification of BVRA in brain promotes insulin resistance in a triple transgenic mouse model of this disorder[101], providing a further connection between oxidative damage and glucose dysmetabolism in the brain.

Many statins, including atorvastatin, do not extensively penetrate the blood–brain barrier (BBB), yet atorvastatin use is associated with lowered risk of developing AD[113,114]. Atorvastatin given to aged beagle dogs protected against oxidation of BVRA, and this statin increased HO-1 levels, whereas oxidative damage levels in the brain were lowered[114]. These studies suggest that following atorvastatin use, some moiety in the periphery is able to penetrate the BBB to target the HO-1–BVRA system or other systems to protect the brain. It also suggests that atorvastatin, although not a direct antioxidant and not able to cross the BBB, leads to antioxidant-like effects in the brain[100]. There is also growing interest in agents (which may include polyphenols) that affect the gut microbiome to exert neuroprotection, but that is beyond the scope of this Review.

Concluding remarks

The evidence for oxidative damage being a critical component of the pathology and progression of AD is compelling[8,11,115]. Glucose metabolism, a key source of energy for the brain, is defective in PCAD, aMCI and AD. This defect likely results in substantial part from oxidative damage to key proteins in glycolysis, the TCA cycle and ATP synthase. Other proteins are also oxidatively damaged, such as BVRA, UCHL1 and the proteasome. Decreased ATP results in numerous changes in brain function, such as impaired maintenance of membrane potentials and increased intracellular Ca^{2+} levels, leading to detrimental downstream effects on cell function and survival.

Small oligomers of Aβ42 solubilize in the membrane to promote oxidative damage, such as lipid peroxidation. This produces reactive aldehydes (TABLE 2) that bind to critical brain proteins to change their conformation and to lower their activity in aMCI and AD brains. Presynaptic membranes are particularly vulnerable to such oxidative damage, leading to diminished learning and memory and ultimately dementia. Subsequent neuronal death also contributes to the clinical presentation and pathology observed in aMCI and AD. Clinical trials in both conditions using antioxidants have been disappointing, but better design of such trials combined with the use of appropriate biomarkers of disease progression and of oxidative damage[8] (to ensure that the antioxidants are actually decreasing it) offers hope[2,8,92]. As AD neuropathology begins at least two decades before symptoms appear, increased efforts to discover better biochemical (for example, plasma) and/or imaging biomarkers for the presymptomatic presence of AD are essential. Given the expected large increase in individuals with AD as the world's population of aged individuals grows and diabetes becomes more frequent, better therapeutic approaches to preserve and/or improve glucose utilization, decrease oxidative damage and protect the brain against neuropathological changes will be needed.

The overall hypothesis presented in this Review for the pathogenesis and progression of AD is that Aβ42 oligomer-induced oxidative stress impairs glucose metabolism, leading to synaptic dysfunction and eventual neuronal death (demonstrated by thinning of key brain areas), ultimately causing aMCI, PCAD and AD. A recent paper is highly consistent with this sequence of events[116]. The authors employed various imaging modalities using subjects from the DIAN (see above) who had been regularly scanned from as long as 22 years before to 3 years after the onset of symptoms using ^{11}C-PiB (Pittsburgh compound B; a positron emission tomography (PET) ligand that detects Aβ aggregates), ^{18}F-fluorodeoxyglucose (to determine glucose metabolism) and MRI (to determine thickness of various brain regions). The authors demonstrated that the first detectable pathological change in asymptomatic persons in the DIAN is deposition of Aβ aggregates (which would imply membrane-resident oligomers that would lead to oxidative damage), followed by decreased glucose metabolism and finally thinning of key brain areas[116]. Continued studies interrogating this sequence of changes should lead to a greater understanding of the pathogenesis and progression of AD and better means to monitor the therapeutic efficacy of promising new agents directed against the production of Aβ, oxidative stress, impaired glucose metabolism and neuronal death. Other cohorts such as AIBL and ADNI will also continue to contribute valuable insights in this context[2,75,117]. This era is one of exciting new developments in aMCI and AD research and potential therapeutic modalities. We both look forward to contributing to this future.

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