Lecture 2c - Mitochondrial involvement in neurodegenerative disease

- Brain vulnerabilities: a lot can go wrong!
- Mitochondrial features of neurodegenerative disease
- Effect of metabolic disease on neurodegenerative disease
- Alzheimer's disease
- [Lectures from practitioners: Dr Jenny Goodman (AD and MS), Lucille Leader and Gilian Crowther (PD)]

Brain vulnerabilities (1)

Brain cells have particular vulnerabilities over and above those of other cells.

- **Excitotoxicity**: Neurons suffer damage or death from **pathologically high levels of neurotransmitters** (particularly glutamate and N-Methyl-D-aspartate - NMDA), resulting in excessive stimulation of receptors, massive influx of calcium, mitochondrial depolarisation and ROS production.
- **Iron dysregulation**: Excess iron in the brain has a 'rusting' effect. In **AD** iron has been found in amyloid β plaques, neurofibrillary tangles and activated microglia and in **PD** with iron accumulation in the substantia nigra, where it correlates with 'freezing of gait', severity of motor symptoms and dopaminergic neurodegeneration. Ferritin levels were associated with cognitive performance in those with MCI and in healthy subjects who carry the Apo E4 allele. We know that excess brain iron has pathogenic properties because **iron chelators can improve symptoms of AD, ALS and PD**.
- **Copper dysregulation**: contributes to neuronal death in Wilson's disease and has been linked to neurodegeneration and the pathogenesis of Alzheimer's and prion diseases.

Brain vulnerabilities (2)

- **Elevated intracellular calcium through MAM dysfunction**: If intracellular calcium is too high, the protein motors that drive mitochondria around the neuron on the cytoskeletal tracks (microtubules) are switched off.
- **Misfolded proteins**: proteins aggregate and stick to other proteins, impeding action. They are characteristic of neurodegenerative disease and can greatly compromise neuronal mitochondrial function and movement.
  - **Misfolded proteins include tau and β-amyloid in Alzheimer's disease, α-synuclein in Parkinson's disease and prions in mad cow disease** (Creutzfeldt-Jakob disease, CJD).
  - Prions are a particular type of misfolded protein with the **ability to transmit their misfolded shape onto normal proteins of the same type, known as 'seeding',** by forcing normal copies of a protein into the same self-propagating, misfolded shape**. This in effect makes the misfolding 'infectious'.**
  - Does this make AD, PD and possibly ALS prion diseases? Possibly. Researchers now believe that many neurodegenerative diseases may be prion diseases and have identified specific proteinaceous infectious particles linked to these diseases, and that have noted that AD behaves like a slow-moving version of CJD. (Weickenmeier J, J Mechanics Physics Solids, 2019; https://royalsocietypublishing.org/doi/10.1098/rsif.2019.0356).
  - There is also some indication that the vaccine-induced SARS-CoV-2 spike protein may also be a prion. Stephanie Seneff has warned that α-synuclein can get drawn into misfolded spike proteins, setting the scene for increased incidence of PD.
  - About one-third of the proteins made by the body on any given day are misfolded. However, as we have already seen, heat-shock proteins (HSPs) can ensure the misfolding is corrected, or if too severe, then the proteins are removed. In this sense, HSPs act as agents of autophagy. Fortunately HSPs can be induced: specifically by alternating extreme hot and cold.
- Note: Many of the cellular processes that are pathogenic in AD are also pathogenic in PD but in different brain areas: hippocampus for AD; substantia nigra for PD (Mattson MP, Ann NY Acad Sci, 2006).

Brain vulnerability: neuronal energy requirement and antioxidant status

- The requirement for energy, and hence mitochondrial ATP production, is much greater in the brain than in other organs. The brain is responsible for 20% of all energy expenditure, although it accounts for only 2% of body weight. Cortical neurons require approximately 4.7 billion ATP per second to ensure continuous function; without this they die.
- In particular, neurodegenerative disease has been associated with a slowing of the motors which help mitochondrial movement in neurons.
- But the brain is particularly vulnerable to free radical damage because of its rich oxygen supply and high fatty acid content in cell membranes. So one would think that the brain's antioxidant supply is plentiful….but it isn't. The brain is highly susceptible to oxidative damage and hence mitochondrial dysfunction, leading to a slow accumulation of lipid and other oxidative damage over time.

(Ravera S, Int J Biochem Cell Biol, 2009; Zhu XH, Neuroimage. 2012; 60(4):2107-17; Nicholls DG, Physiol Rev, 2000; 80(1):315-60; Sheng ZH, Nat Rev Neurosci, 2012; 13(2):77-93)

Brain vulnerability: excitotoxicity

- Excitotoxicity can occur when neurons suffer damage or death from pathologically high levels of neurotransmitters (particularly glutamate and NMDA), resulting in excessive stimulation of receptors.
- When there is prolonged opening of the receptor for N-methyl-D-aspartate (NMDA) at the plasma membrane (e.g., during brain ischaemia), this results in a massive influx of calcium which is taken up into mitochondria, producing a profound and irreversible mitochondrial depolarisation. Calcium-induced neurotoxicity is thought to be a key mechanism of cell death in neurodegenerative disease, often via mtPTP opening.
- Excitotoxicity causes a continual high demand for mitochondrial energy production, but the additional ATP cannot be utilised by neurons. This mismatch of demand and supply creates free-radical production and eventually cellular apoptosis.
- Damage to the mitochondria in the early stages of excitotoxicity includes fission-induced fragmentation, an indication that the neuron is under intense stress. This leads to small, fragmented and vulnerable mitochondria and is accelerated by nitric oxide from neuronal nitric oxide synthase, a calcium-dependent enzyme.

(Burchell VS, Expert Opin Ther Targets, 2010; Martorell-Riera A, Cell Cycle, 2015)

Brain vulnerability: iron dysregulation

- In Alzheimer's disease (AD) patients, iron can aggregate with amyloid β plaques, neurofibrillary tangles and activated microglia; in the most severe cases, iron accumulated along myelinated fibres. Cortical iron was associated with increased cognitive decline in AD patients and in older adults. Iron levels correlated with the amount of amyloid plaque and tau pathology. AD patients had increased hippocampal ferritin levels, while mild cognitive impairment (MCI) was associated with higher levels of the soluble transferrin receptor (involved in iron uptake). Yet AD patients with excess neuronal iron had low serum Fe (and lower Zn).
- In AD patients, the hippocampus, which is the brain area most damaged in AD, contained increased ferritin iron (Raven EP, J Alzheimers Dis. 2013). Ferritin levels were associated with cognitive performance in those with MCI and in healthy subjects who carry the Apo E4 allele (Ayton S, JAMA Neurol. 2017). Iron chelators have been shown to help patients with AD (Liu JL, Front Neurosci. 2018).
- In Parkinson's disease (PD) there is risk of iron accumulation in the substantia nigra; this correlates with 'freezing of gait', severity of motor symptoms and dopaminergic neurodegeneration. The combination of dopamine and iron generated ROS, which resulted in protein misfolding. In dopaminergic neurons, loss of the transferrin receptor 1 caused neuronal iron deficiency, age-progressive degeneration of dopaminergic neurons and motor deficits, damaged mitochondria accumulated, and gene expression signatures indicated attempted axonal regeneration, a metabolic switch to glycolysis, oxidative stress, and the unfolded protein response.
- Iron metabolism may also be dysregulated in amyotrophic lateral sclerosis (ALS), with serum ferritin elevated in patients.
- We know that excess brain iron has pathogenic properties because iron chelators can improve symptoms of AD, ALS and PD.
- A US Framingham serum study showed that 3% of the elderly were iron-deficient but 13% had iron overload.
- Studies are divided over whether serum Fe is higher or lower in PD patients (but it is definitely not in reference range). So any iron imbalance is going to cause a problem in neurons. As the neuroscientist JR Connor put it 'Life was designed to exist at the very interface between iron sufficiency and deficiency'.
- At the cellular level, iron can react with hydrogen peroxide in the inner mitochondrial membrane. This is a normal part of cellular aerobic respiration. However, excess iron catalyses the formation of too many damaging hydroxyl free radicals from peroxide. There is a specific form of apoptosis called ferroptosis, which is dependent on and regulated by iron. Dysregulated ferroptosis is seen in cancer cells, while ferroptosis upregulation is being trialled as a cancer treatment. Mitochondrial ferritin can lower the cellular iron pool and protect against hydrogen peroxide-induced oxidative stress and apoptosis in neurons by inhibiting ROS production, maintaining mitochondrial membrane potential and inhibiting apoptosis.

(Isaya G, Front Phamacol, 2014; Mena NP, Mitochondrion, 2015; Raven EP, J Alzheimers Dis, 2013; .Van Duijn S, J Alzheimer's Dis, 2017; Ayton S, Mol Psychiatry, 2019; Xu J, Medicine, 2019; Matak P, Proc Natl Acad Sci USA, 2016; Kristinsson J, Neuropsychiatr Dis Treat, 2012; Martin-Bastida A, Eur J Neurol, 2017; Arrequin S, J Inorg Biochem, 2009; Naduthota RM, J Neurol Sci, 2017; Qureshi M, Open Neurol J, 2008; Devos D, J Neural Transm, 2020; Tao Y, J Alzheimer's Dis, 2014; Li DD, Front Aging Neurosci, 2017; Fleming DJ, Am J Clin Nutr, 2001; Mou Y, J Hematol Oncol. 2019; Gao G, Aging Dis. 2017)

Brain vulnerability: copper dysregulation

- Copper toxicity contributes to neuronal death in Wilson's disease and has been linked to neurodegeneration and the pathogenesis of Alzheimer's and prion diseases.
- Exposure of astrocytes to copper led to rapid depolarisation of the mitochondrial membrane potential and increased ROS production. This led to reduced cell viability, respiratory function and mitochondrial membrane potential, with increased ROS production in a rat cell line.
- Copper catalysed an increase in hydroxyl radical generation in solution and its addition to murine neocortical cell cultures induced a decrease in ATP levels and neuronal death through activation of caspase 3; free radical scavengers inhibited apoptosis, indicating that the effect was due to oxidative damage.
- Mitochondrial electron transport was dose and time dependently reduced by copper and manganese

(Sheline CT, Ann Neurol, 2002; Gyulkhandanyan AV, J Neurochem, 2003; Rossi L, Neurochem Res, 2004; Heron P, Life Sci, 2001; Reddy PV, Lab Invest, 2008)

Brain vulnerability: misfolded proteins

- Many proteins need to be folded to enable them to function appropriately, but when they are misfolded, they clump together to form aggregates that stick to other proteins, impeding action.
- Misfolded proteins are a characteristic of neurodegenerative disease and can greatly compromise mitochondrial function and movement within the neuron. Misfolded proteins include tau and β-amyloid in Alzheimer's disease, α-synuclein in Parkinson's disease and prions in mad cow disease (Creutzfeldt-Jakob disease, CJD).
- Hallmarks of sporadic CJD include neuron loss, synaptic decline and spongiform change; defects in all 5 ETC Complexes and altered purine metabolism were seen in CJD cases at post mortem.
- Misfolded proteins spread their pathology through aggregation and intercellular propagation, known as 'seeding', by forcing healthy proteins to misfold in a cascade of damage, in effect acting as an infectious agent. This is what is feared when eating meat infected with CJD – that the infection will be 'seeded' in the brains of those consuming it.
- Correct protein folding requires high levels of oxidised glutathione.

(Angelova PR, Biophys Biochem Res Commun, 2016; Golde TE, J Clin invest, 2013; Vickers JC, Brain Res Bull, 2016; Ansoleaga B, J Neuropathol Exp Neurol, 2016; Falcon B, J Biol Chem, 2015; Perez-Torres I, Int J Mol Sci, 2017; Guardia-Laguarta C, J Neurosci, 2014; Schon M, J Alzheimers Dis, 2010; Tambini MD, EMBO Rep, 2016)

Could other neurodegenerative conditions be prion diseases? Yes, possibly

- Researchers have noted that Alzheimer's disease (AD) behaves like a slow-moving version of CJD. Prions (misfolded proteins, which may be infectious) are considered a subclass of amyloids in which protein aggregation becomes self-perpetuating and infectious. So far there is no evidence of transmission between individuals or between species but researchers are concerned. Why?
- Amyloid-β (Aβ) pathology was found in 50% of CJD patients in post-mortem investigations. Also, research published in 2011 found a prion-like protein in 25-50% of Alzheimer's patients, as well as Parkinson's and ALS patients. Research presented in 2014 revealed that Alzheimer's patients with this protein were 10 times more likely to have been cognitively impaired at death than those without it and the presence of the prion-like protein is positively correlated with early-onset AD. Furthermore, both Aβ and tau act as prions, effectively making AD a potential double-prion disease.
- When tiny amounts of Aβ proteins are injected into animals, they act as self-propagating 'seeds'," unleashing a chain reaction of protein misfolding that results in pathology that is very reminiscent of that seen in Alzheimer's patients.
- Parkinson's disease is also thought to be a prion disease, resulting from increased production and/or impaired clearance of α-synuclein.

(Jucker M, Nature, 2013; Sabate R, Prion, 2014; Wilson AC, Int J Clin Exp Pathol, 2011; Aoyagi A, Sci Transl Med, 2019; Jaunmuktane Z, Nature, 2015; Wilson AC, Int J Clin Exp Path, 2011; Olanow CW, Mov Disord, 2013; Chauhan A, Neuro Res Int, 2015)

Intracellular calcium and mitochondria in neurodegeneration

- Mitochondria are a major site of calcium storage in neurons and the storage capacity increases with potential across mitochondrial membranes.
- Most of the problems of excess intracellular calcium have been identified in neurodegenerative disease, but may apply to other disease conditions:
  - Problems with the mitochondria-associated endoplasmic reticulum membrane (MAM) have been identified in PD and AD.
  - If intracellular calcium is too high, the protein motors that drive mitochondria around the cell on the cytoskeletal track (microtubules) are switched off. This is of particular concern in neurons, which have long axons.
  - In ALS (amyotrophic lateral sclerosis, aka motor neurone disease), loss of motility of mitochondria along the neurons has been linked with elevated intracellular calcium.
  - Excitotoxicity: the neurotransmitter glutamate acts on neuronal NMDA (N-methyl-D-aspartate) receptors to allow calcium influx to increase neuronal excitotoxicity, which leads to elevated intracellular calcium. This leads to damaged mitochondria as they take up large amounts of the excess calcium to protect the cell, but can become overwhelmed. Following fission-induced fragmentation, mitochondrial damage results in opening of the PTP and loss of the mitochondrion and eventually of the whole neuron.

Intracellular calcium and mitochondrial uncoupling proteins in neurons

- UCPs 2, 4 and 5 can affect intracellular calcium.
- UCP2 appears to be neuroprotective by reducing mitochondrial calcium uptake and preventing mitochondrial ROS accumulation following cerebral ischaemia.
- UCP 4 and 5 can alter cellular calcium levels. Neurons in the hippocampus experience increased concentrations of ATP in the presence of these uncoupling proteins, suggesting that UCPs can improve synaptic plasticity and transmission.
- When uncoupling proteins reduce the potential across mitochondrial membranes, calcium ions are released to the surrounding environment in the neuron. The high concentrations of mitochondria near axon terminals, suggests that UCPs play a role in regulating calcium concentrations in this region and may directly affect neurotransmission.

Is it all bad news for the brain? No.

- We have a **glymphatic system**, which clears waste from the central nervous system (CNS), mostly during sleep. It is the brain equivalent of the lymphatic system. (Benveniste H, Gerontology, 2019; Hauglund NL, Curr Opin Physiol, 2020; Jessen NA, Neurochem Res, 2015)

- And we have **neural stem cells**. It used to be thought that we were born with all the brain cells we will ever have. But a recent post-mortem study investigating the hippocampus, the area for memory and emotion, showed that we keep making new brain cells throughout our lives (Llorens-Martin M, Exp Neurol, 2015).

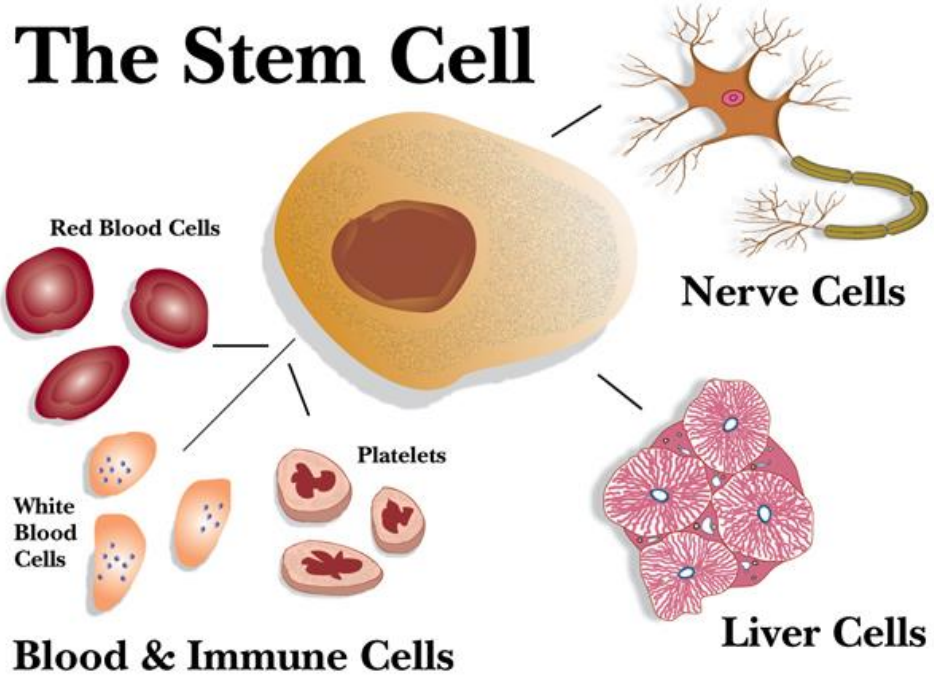
We have a glymphatic system

- The glymphatic system is a network of vessels formed by astroglial cells, that clear waste from the central nervous system (CNS), mostly during sleep. It is the brain equivalent of the lymphatic system.
- The glymphatic system performs an intra-brain detox and overnight brain maintenance. It sends clear cerebral spinal fluid through brain tissue, flushing out cellular waste and neurotoxins and transporting them into the circulation to be processed as waste in the liver.
- Brain cells shrink up to 60% as we sleep to increase the interstitial space and make it easier for glymphatic fluid to circulate through brain tissue; after 'washing', the brain cells return to normal size.
- This takes a lot of energy, which is why it takes place at night. But for the glymphatic system to work optimally, we must have adequate deep sleep. The biological need for sleep across all species may therefore reflect the brain's need to eliminate potentially neurotoxic waste products, including β-amyloid.
- Recent evidence suggests that the glymphatic system may be disrupted in some diseases of the brain, leading researchers to speculate that disruption may contribute to cognitive decline.

(Jessen NA, Neurochem Res, 2015; Benveniste H, Gerontology, 2019; Hauglund NL, Curr Opin Physiol, 2020)

We have neural stem cells

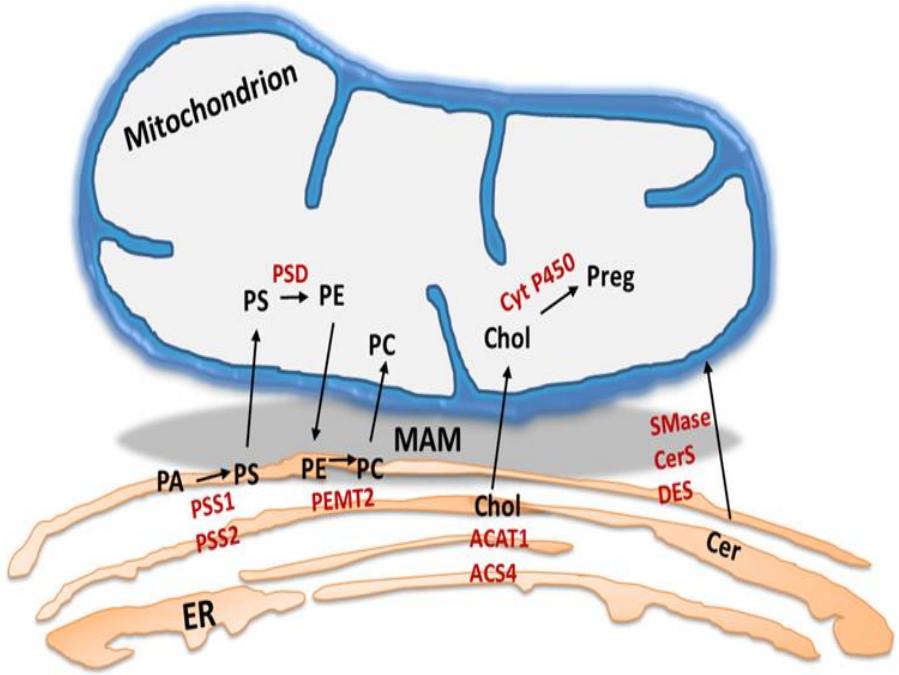
- It used to be thought that we were born with all the brain cells we will ever have. But a recent study of 58 deceased subjects aged 43-97, investigating the hippocampus, the area for memory and emotion, showed that we keep making new brain cells throughout our lives. But the rate of renewal does tail off with age and is much lower in Alzheimer's disease patients.
- Neural stem cells (NSCs) are self-renewing, multipotent cells that firstly generate the radial glial progenitor cells that generate the neurons and glia of the nervous system during embryonic development.
- Some neural progenitor stem cells persist in highly restricted regions in the adult vertebrate brain and continue to produce neurons throughout life, playing an important role in adult learning and hippocampal plasticity. Neural stem cells have been shown to engage in migration and replacement of dying neurons and can modulate neurogenesis in mature neurons through chemical signals acting on neural stem cells.



(Llorens-Martin M, Exp Neurol, 2015; Beattie R, FEBS Lett, 2017; Pardal R, Dev Growth Differ, 2016)

Mitochondrial features of neurodegenerative disease

- Neurodegenerative diseases show **all the usual characteristics of mitochondrial dysfunction plus misfolded proteins and failure to degrade damaged proteins**. Synaptic mitochondria have particularly poor ATP production due to inhibition of ETC Complexes.
- **Many of the problems implicate the MAM (mitochondria-associated endoplasmic reticulum membrane), through poor calcium ion regulation**; dysregulation is commonly seen in Alzheimer's and Parkinson's diseases. In fact, α-synuclein can sabotage calcium homeostasis between the endoplasmic reticulum and the mitochondria.
- **The ApoE4 genotype**, seen in Alzheimer's and cardiovascular disease patients, **increases activity of the MAM, resulting in additional calcium ion dysregulation and release of mtDNA and cardiolipin into the cytosol**, which can trigger an inflammatory reaction.



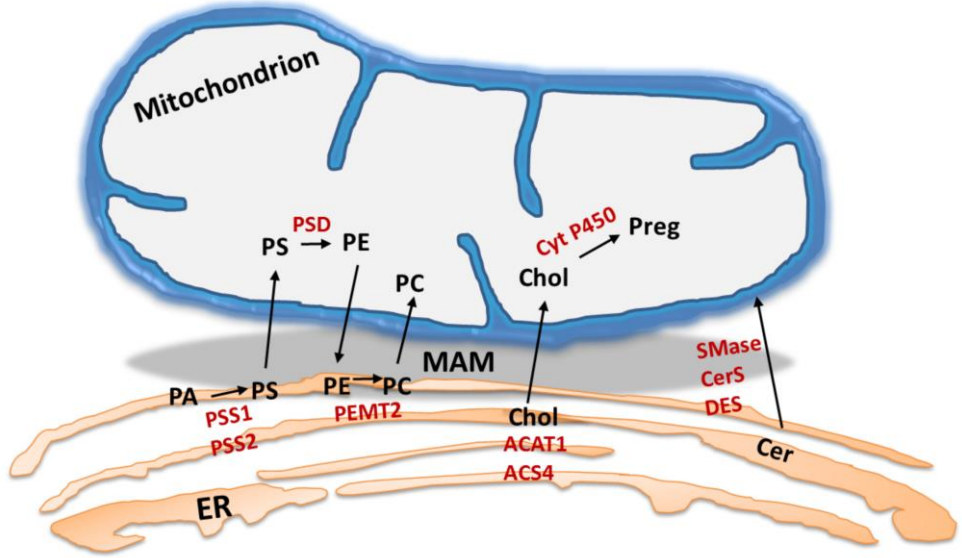
Mitochondrial features of neurodegenerative disease

- Neurodegenerative diseases show all the usual characteristics of mitochondrial dysfunction plus poor protein folding and failure to degrade damaged proteins. Synaptic mitochondria have particularly poor ATP production due to inhibition of ETC Complexes. Many of the problems implicate the mitochondria-associated endoplasmic reticulum membrane (MAM), see next slide.
- Poor intracellular calcium homeostasis, increased autophagy (generating neuronal loss and increased ROS), iron-sulphur complex deficiency and excess iron tend to be more pronounced in neurons and are associated with neurodegenerative disease incidence.
- Neuronal stress, such as excitotoxicity or ischaemia, cause over-activation of the DNA repair enzyme, PARP1, which decreases cytosolic NAD+ and increases neuronal ROS production and expression of mitochondrial SIRT3. Overexpression of SIRT3 suppresses ROS generation and prevents apoptosis, thereby preventing neuronal death.
- Although each neurodegenerative disease is very different, there is a striking similarity in a number of characteristics: mitochondrial condition, neuronal death, abnormal accumulation of misfolded proteins and oxidative stress. Furthermore, almost identical disease phenotypes are seen both in sporadic forms (risk increases progressively with age) and in heritable familial forms (mutations of specific genes that usually occur in a younger age group.
- The same characteristics are also seen in acute central nervous system disorders, such as stroke. The loss of cells is amplified by the limited regenerative abilities for cell replacement and repair in the CNS.

(Rigotto G, Oxid Med Cell Longev, 2019; Burchell VS, Expert Opin Ther Targets, 2010; Wilson TJ, Neurobiol Dis, 2013; Nissanka N, FEBS Letts, 2018; Akhter F, Prog Mol Biol Transl Sci, 2017; Davey GP, J Biol Chem, 1998; Sripetchwandee J, Front Endocrinol, 2018; Duchen MR, Eur J Physiol, 2012; Ganguly G, Drug Des Devel Ther, 2017; Kamat PK, Cell Biochem Biophys, 2014; Batlevi Y, Neurobiol Dis, 2011; Isaya G, Front Phamacol, 2014; Mena NP, Mitochondrion, 2015; Ansari A, Aging Cell, 2017)

The mitochondria-associated endoplasmic reticulum membrane (MAM) in neurodegenerative disease

- The mitochondrial-associated endoplasmic reticulum membrane (MAM) is crucially connected to the pathology of neurodegenerative disease through poor intracellular calcium regulation and misfolded proteins and dysregulation is commonly seen in Alzheimer's and Parkinson's diseases. In fact, α-synuclein can sabotage calcium homeostasis between the endoplasmic reticulum and the mitochondria.
- Two of the potentially misfolded proteins, β-amyloid and α-synuclein (in Alzheimer's and Parkinson's, respectively) have links to the MAM; researchers are developing a MAM hypothesis for both conditions.
- The ApoE4 genotype, seen in Alzheimer's and cardiovascular disease patients, increases activity of the MAM, resulting in mitochondrial stress, resulting in release of mtDNA and cardiolipin into the cytosol, which can trigger an inflammatory reaction.



Metabolic disease and neurodegenerative disease

- **AD has been called type 3 diabetes, for good reason.**
- **T2D has been associated with a 50% increased risk of AD.**
- **But curiously, despite the excess glucose, T2D manifests as a brain energy deficit, which is an important pre-symptomatic feature of AD** and could be developed diagnostically. Glucose uptake is lower in the frontal cortex of those >65 years-old despite normal cognitive scores, and also in adults <40 years-old at risk for AD. The impaired brain glucose uptake is due to structural atrophy, although there is no such impaired brain uptake of ketones.
- **T2D is also associated with vascular dementia, PD and Huntington's disease. Even T1D is also associated with increased risk of dementia.**
- Obesity, insulin resistance and T2D have long been considered risk factors for the development and progression of late onset AD; obesity alone can lead to brain shrinkage. Alzheimer's-type neuropathological changes were frequent in morbidly obese elderly individuals without clinical history of cognitive impairment.

Correspondence between metabolic and neurodegenerative disease (not just Alzheimer's)

- Obesity, insulin resistance and T2D have long been considered risk factors for the development and progression of late onset AD. Obesity alone can lead to brain shrinkage, with smaller subcortical gray matter volumes; this is the area of the brain that handles high level brain functions. Alzheimer's-type neuropathological changes were frequent in morbidly obese elderly individuals without clinical history of cognitive impairment, approaching those seen in Alzheimer's disease. Higher incidence of diabetes, insulin resistance, fasting insulin and HbA1c (the long term measure of blood glucose) were all associated with brain volume shrinkage. However, voluntary weight loss was associated with higher cognitive brain function.
- A large US study found that higher glucose levels may be a risk factor for dementia, even among persons without diabetes. AD has been called type 3 diabetes, for good reason. T2D has been associated with a 50% increased risk of AD and the risk associated with the APOE 4 allele is exacerbated by diabetes. A Mayo Clinic study reported that >80% of AD patients exhibit T2D or abnormal blood glucose levels. However, there is also a risk of AD death in patients with low blood glucose.
- T2D is also associated with vascular dementia, PD and Huntington's disease but even among people without diabetes, high levels of glucose or HbA1c are risk factors for impaired cognitive function and dementia. Advanced glycation end products (AGEs) are found in both diabetic and AD patients.
- T1D is also associated with increased risk of dementia through alterations in brain structure and function, although the association with T2D is stronger. Altered neuronal insulin signalling has molecular and biochemical features that overlap with T1D and T2D.
- Mitochondria and are thought to mediate the susceptibility to neurodegenerative diseases in T2D, possibly through MAM-related pathology.

(Dekkers IA, Radiology. 2019; Debette S, Ann Neurol, 2010; Hamer M, Neurology, 2019; Mrak RA, Clin Neuropathol, 2009; Ronan L, Neurobiol Aging. 2016; Tan ZS, Diabetes Care. 2011; Neseliler S, Cell Metab, 2019; Crane PK, New Eng J Med, 2013; Rigotto G, Oxid Med Cell Longev, 2019; Baker LD, Arch Neurol, 2011; De Felice FG, Diabetes, 2014; Moreira PI, J Alzheimers Dis, 2018; Sripetchwandee J, Front Endocrinol, 2018; Yu Q, J Alzheimers Dis, 2016; Schon EA, J Alzheimers Dis, 2010; Tubbs E, Diabetes, 2014; De la Monte SM, J Diabetes Sci Technol, 2008; Willette AA, JAMA Neurology, 2015; Zhang F, Diabetologia, 2018; Ma Y. Int J Epidemiol. 2020; 49(4): 1353–1365; Steen E, J Alzheimer's Dis. 2005)

Alzheimer's disease and brain energy sources

- But a brain energy deficit is an important pre-symptomatic feature of AD. Glucose uptake is lower in the frontal cortex of those >65 years-old despite normal cognitive scores, and also in adults <40 years-old at risk for AD, including the apolipoprotein E4 allele, insulin resistance and T2D.
- Brain glucose uptake is impaired in AD and mild cognitive impairment due to structural atrophy, but brain uptake of ketones remains the same in AD and MCI as in cognitively healthy age-matched controls.
- Increasing ketone availability to the brain via moderate nutritional ketosis has a modest beneficial effect on cognitive outcomes in mild-to-moderate AD and in mild cognitive impairment, suggesting that it may correct several deficiencies that are associated with glucose hypometabolism
- ApoE2 and ApoE4 brains showed a similar level of ability to metabolise ketones, whereas ApoE3 brains presented a relatively deficient profile.

(Croteau E, Exp Gerontol, 2018; Cunnane SC, Front Mol Neurosci, 2016; Cunnane SC, Ann N Y Acad Sci, 2016; Wu L, J Neurosci, 2018; Pawlosky RJ, Int J Mol Sci, 2020; Pawlosky RJ, J Neurochem, 2017)

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Medical News & Perspectives

In Alzheimer Research, Glucose Metabolism Moves to Center Stage

Bridget M. Kuehn, MSJ

Areas or patterns of reduced glucose metabolism are often seen in brain scans of patients with Alzheimer disease and other dementias. Now, a growing body of evidence suggests that glucose hypometabolism may be more than just a biomarker on brain scans: it may evated risk of developing Alzheimer disease and other forms of dementia. But the nature of this relationship isn't clear.

"We've known for probably 20-plus years that type 2 diabetes seems to increase risk for Alzheimer disease," Macauley said. "We're still trying to understand why." currently studying how, but he suspects that diabetes and prediabetes affect the brain through multiple pathways, some of which may be improved by drug therapy, including neurovascular insult.

"At the very least, diabetes creates a hit that makes the brain more susceptible to

It’s not all straightforward: Brain insulin is neuroprotective but glucose is neurotoxic

- Insulin in the brain not only comes from pancreatic β-cells but can also be synthesised in neurons of the cortex and hippocampus; insulin receptors have been detected in several brain areas.
- The role of brain insulin is not primarily involved with cellular glucose uptake but with promoting synaptic and neuronal plasticity and mediating cognitive function and memory processes. It also has a neuroprotective effect through inhibition of apoptosis and Aβ production and induction of antioxidant production.
- Administration of insulin showed positive effects on learning and memory, as assessed by improvement in brain synaptic plasticity, and was related to activation of insulin receptors and downstream signalling, although insulin administration in APOE 4 carriers seems to exacerbate cognitive deficits.
- Several classes of non-insulin hypoglycaemic agents such as metformin and glitazones, which stimulate PPAR-γ, can successfully treat both T2D and AD, improving neuronal glucose utilisation, brain insulin sensitivity and hippocampal synaptic plasticity. Again, improvement was not seen in APOE4 carriers. The use of GLP-1 analogues (liraglutide, exendin-4) increase brain insulin, insulin-like growth factor 1 and GLP-1 signalling and decrease phosphorylated tau levels and apoptosis.
- Insulin degrading enzyme (IDE) breaks down insulin to prevent brain hypoglycaemia and it also helps to destroy amyloid plaques. But it can’t do both at the same time! So we need sufficiently low brain glucose that the IDE can break down amyloid plaques as appropriate.

(Talbot K, J Clin Invest, 2012; Arnold SE, Nature Rev Neurol, 2018; Rigotto G, Oxid Med Cell Longev, 2019; Sripetchwandee J, Front Endocrinol, 2018; Carvalho C, Adv Exp Med Biol, 2019; Abolhassani N, Mech Ageing Dev, 2017; Sripetchwandee J, Front Endocrinol, 2018; Burchell VS, Expert Opin Ther Targets, 2010; Abolhassani N, Mech Ageing Dev, 2017; Moreira PI, J Alzheimers Dis, 2018; Ahmad D, Mol Neurobiol, 2013; Farris W, PNAS. 2003; 100(7): 4162-7)

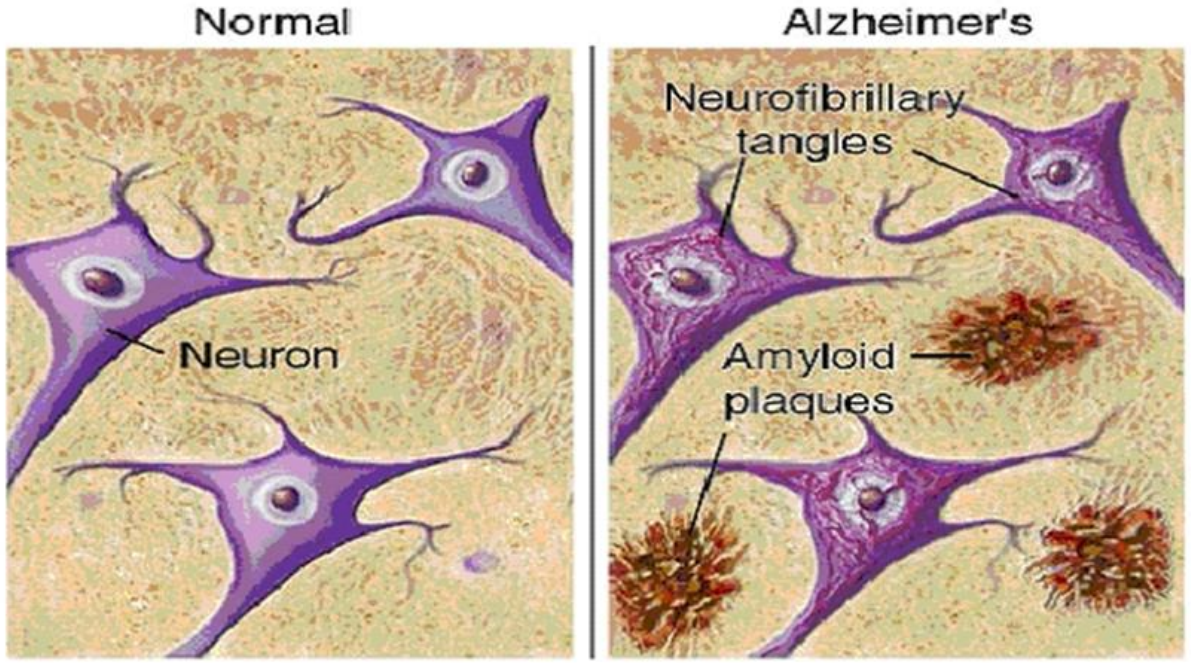
Alzheimer's disease (AD): incidence rapidly multiplying

- Described as the biggest health challenge of modern times, the global number of people living with dementia more than doubled from 1990 to 2016 and the WHO estimates of the global number who will have dementia by 2050 have risen to 150 million.
- In the UK, over 500,000 have been diagnosed with AD (so the actual incidence is probably much higher. The current cost burden of dementia is £26 billion; it is projected to rise to £59 billion by 2050.
- In the US, deaths from AD have risen 145% between 2000 and 2017; it is the 3rd leading cause of death, behind cancer and heart disease. Estimated care costs run at $500bn per year.
- The US CDC reports that in 2020, 5.8 million Americans were living with Alzheimer's disease; the number of people living with the disease doubles every 5 years beyond age 65. This number is projected to nearly triple to 14 million people by 2060. Those with early onset dementia have increased by 200% since 2013.

(GBD Dementia Collaborators 2016, Lancet Neurol, 2019; https://apps.who.int/gho/data/node.dementia; https://www.ohe.org/publications/trajectory-dementia-uk-making-difference)

Alzheimer's disease (AD) characteristics

- **AD is characterised by the presence of amyloid beta (Aβ) and neurofibrillary tangles (NFTs), formed by hyperphosphorylated tau protein.**
- Aβ is a peptide deriving from amyloid precursor protein (APP). Aβ molecules can aggregate and may become misfolded and induce other Aβ molecules to also take the misfolded oligomeric form, which is toxic to neurons.
- Aβ is seen in 2 forms: soluble Aβ1-40 (or Aβ40) and insoluble Aβ1–42 (or Aβ42).
- NFTs may also form misfolded protein oligomers.



(Oliver DMA, J Alzheimers Dis, 2019; Grimm A, Biogerontology, 2016; Hiltunen M, J Alzheimers Dis, 2009; Fernández-Moriano C, Oxid Med Cell Longev, 2015)

Alzheimer's is not just a prion disease – it is a double prion disease

- **Both amyloid β and tau proteins show prion tendencies, making AD a double prion disease.**
- In a ***post mortem* study** of 75 AD patients, self-propagating prion forms of Aß and tau proteins were detected. **Higher levels of these prions were strongly associated with early-onset forms of the disease and younger age at death**; self-propagating prion forms of tau and Aß were most infectious in the brains of Alzheimer's patients who died at a young age from inherited, genetically driven forms of the disease, but much less prevalent in patients who died at a more advanced age. The quantity of tau prions in the brain of a patient who died at age 40 were on average 32 times higher than in a patient who died at 90. **It was prion activity that correlated with disease severity, rather than the amount of plaques and tangles at the time of autopsy.** (Aoyagi A, Sci Transl Med, 2019)
- There is **little indication that prions are implicated in late-onset AD**, where patients die at a normal age.
- For a number of years, researchers have postulated and found evidence that suggests Alzheimer's disease may be a type of prion-based disease. (Zhou J, Intractable Rare Dis Res. 2013)
- Scientists have found that Alzheimer's behaves like a slow-moving version of CJD; according to one paper, prions are considered a subclass of amyloids in which protein aggregation becomes self-perpetuating and infectious (Sabate R, Prion. 2015).

Alzheimer’s as a prion disease

- Recent reports have documented rare cases of patients treated with growth hormone derived from human brain tissue, or given transplants of the brain’s protective dura mater, who went on to develop Aß plaques in middle age, long before they should be seen in anyone without a genetic disorder.
- Animal research has also found that when tiny amounts of Aß proteins are injected into animals, they act as self-propagating seeds to unleash a chain reaction of protein misfolding that results in pathology similar to that found in Alzheimer's patients (Jucker M, Nature. 2013).
- The cellular prion protein (PrPC) has newly been identified as a cell surface receptor for Aβ oligomers. PrPC is a cell surface glycoprotein that plays a key role in the propagation of prions, proteinaceous infectious agents that replicate by imposing their abnormal conformation to PrPC molecules. In AD, PrPC acts to transduce the neurotoxic signals arising from Aβ oligomers, leading to synaptic failure and cognitive impairment. Interestingly, accumulating evidence has also shown that aggregated Aβ or tau possesses prion-like activity, a property that would allow them to spread throughout the brain. (Zhou J, Intractable Rare Dis Res. 2013)

Alzheimer's disease (AD): characteristics

- AD occurs in two forms: early-onset familial and late-onset sporadic. Genetic mutations in amyloid precursor protein (APP), presenilin 1 (PS1) and PS2 are associated with familial AD, but this type represents less than 1% of AD. Multiple factors are involved in late-onset AD, including the APOE4 genotype, polymorphisms in several gene loci, T2D, traumatic brain injury, stroke and age-related factors, environmental toxins, increased ROS production and mitochondrial dysfunction.
- AD is characterised by the presence of amyloid beta (Aβ) and neurofibrillary tangles (NFTs), formed by hyperphosphorylated tau protein.
- Aβ is a peptide deriving from amyloid precursor protein (APP); its normal function is not well understood. Aβ molecules can aggregate to form flexible soluble oligomers, which may become misfolded and induce other Aβ molecules to also take the misfolded oligomeric form, which is toxic to neurons. The soluble Aβ1-40 (or Aβ40) circulates in the CSF but can be present in plaques, while the insoluble Aβ1–42 (or Aβ42) is found predominantly in plaques, where it creates oxidative stress and has the ability to produce ROS, mainly hydrogen peroxide, when it reacts with transition metal ions present in plaques.
- Neurofibrillary tangles (NFTs) may also form misfolded protein oligomers. There is some evidence that misfolded Aβ can induce tau to misfold. To fulfil its normal function, tau must be phosphorylated, but the extent of phosphorylation decreases with age. In AD, however, tau is hyperphosphorylated, causing it to form NFTs.

(Oliver DMA, J Alzheimers Dis, 2019; Grimm A, Biogerontology, 2016; Hiltunen M, J Alzheimers Dis, 2009; Fernández-Moriano C, Oxid Med Cell Longev, 2015)

Mitochondria in Alzheimer's disease (AD)

- Studies of AD patients **prior to disease onset show respiratory chain defects, particularly impaired activity of Complex IV** (cytochrome c oxidase) and oxidative damage.
- **The early stages of AD are marked by defects in axonal transport of mitochondria and hence synaptic dysfunction** through lack of mitochondrial transport.
- Several studies show that the **degree of disability in AD correlates with the level of bioenergetic impairment in the brain** and that the extent of APP and Aβ accumulation in the mitochondria correlates with mitochondrial dysfunction.
- **In the AD brain**, both microglia and neuronal **mitochondria produce particularly high levels of free radicals**; post-mortem brains show increased oxidative damage in mtDNA, with lipid peroxidation of neuronal tissue and oxidative modifications of proteins.
- **Hyperphosphorylated tau in NFTs itself inhibits mitochondrial function** by decreasing the activity of ETC Complexes. It can also cause excessive mitochondrial fission and leads to the degeneration of mitochondria and synapses in AD patients.
- **Altered calcium dynamics** occur in cells is seen in AD, particularly an **exaggerated release of calcium from mitochondria into the cytosol, triggering apoptosis**. The calcium abnormalities can be directly linked to the altered tau phosphorylation, amyloid precursor protein processing and synaptic dysfunction that are defining features of AD.

Alzheimer's disease and mitochondria: early features of disease

- Studies of AD patients prior to disease onset show respiratory chain defects, particularly impaired activity of Complex IV (cytochrome c oxidase) and oxidative damage. Imaging studies show increased oxygen consumption relative to the amount of glucose being metabolised. This inefficient use of oxygen and decreased glucose utilisation show that mitochondrial dysfunction in the brain is one of the first observable symptoms of the disease; cellular energy production may therefore be a better indicator of AD severity than presence of amyloid plaques.
- Both APP and Aβ can accumulate in the mitochondria and the extent correlates with mitochondrial dysfunction; early accumulation of Aβ in mitochondria may be an initiating pathological event.
- The early stages of AD are marked by defects in axonal transport of mitochondria, as denoted by the abnormal accumulation of mitochondria within large swellings along dystrophic and degenerating neurites. Since tau proteins normally stabilise and maintain the cytoskeletal tracks, hyperphosphorylation of tau proteins in AD results in their destabilisation and fewer mitochondria reaching the synapses. Synaptic dysfunction through lack of mitochondrial transport is an early pathological feature of AD.

(Tobore TO, Neurol Sci, 2019; Berndt N, Int J Cell Biol; Burchell VS, Expert Opin Ther Targets, 2010; Flannery PJ, Mol Cell Neurosci, 2019; Kamat PK, Cell Biochem Biophys, 2014; Correia SC, Biochim Biophys Acta, 2016; Nissanka N, FEBS Letts, 2018; Fernández-Moriano C, Oxid Med Cell Longev, 2015; Gan X, Biochim Biophys Acta, 2014; Rigotto G, Oxid Med Cell Longev, 2019; Sripetchwandee J, Front Endocrinol, 2018; Choi J, Mitochondrion, 2014)

Alzheimer's disease and mitochondria: later features of disease

- Several studies show that the degree of disability in AD correlates with the level of bioenergetic impairment in the brain.
- In the AD brain, both microglia and neuronal mitochondria produce high levels of ROS, as evidenced by upregulation of NADPH oxidase, the main mediator of microglial ROS. Post mortem brains of AD patients show increased oxidative damage in mtDNA, with lipid peroxidation of neuronal tissue and oxidative modifications of proteins.
- Aβ is responsible for mitochondrial mutations in AD development.
- Hyperphosphorylated tau in NFTs inhibits mitochondrial function by decreasing the activity of ETC Complexes. It can also cause excessive mitochondrial fission and lead to the degeneration of mitochondria and synapses in AD patients.
- Altered calcium dynamics occur in cells from patients with both genetic and sporadic forms of AD, particularly an exaggerated release of calcium from mitochondria. The calcium abnormalities can be directly linked to the altered tau phosphorylation, amyloid precursor protein processing and synaptic dysfunction that are defining features of AD.

Alzheimer's disease and mitochondria: other features of disease

- AD neurons show reduced mitochondrial number, biogenesis and function, with deficiency in a key enzyme in the TCA cycle reducing production of NADH. Excess mitochondrial fission leads to mitochondrial fragmentation and calcium overload, formation of the mtPTP and mitochondria-induced apoptosis. AD brains also show evidence of ROS mediated-injury, with increased levels of malondyaldehyde and the toxic 4-hydroxynonenal (HNE) in the brains and cerebrospinal fluid of AD patients.
- Several studies show that the degree of disability in AD correlates with the level of bioenergetic impairment in the brain.
- In the AD brain, both microglia and neuronal mitochondria produce high levels of ROS, as evidenced by upregulation of NADPH oxidase, the main mediator of microglial ROS. Increased oxidative damage in mtDNA is seen in post-mortem brains of AD patients, with lipid peroxidation of neuronal tissue and oxidative modifications of proteins. Oxidative damage, neuroinflammation, synaptic damage and mitochondrial dysfunction are early events in AD development; oxidative damage may decrease with disease progression.
- Altered calcium dynamics occur in cells from patients with both genetic and sporadic forms of AD, particularly an exaggerated release of calcium from mitochondria. The calcium abnormalities can be directly linked to the altered tau phosphorylation, amyloid precursor protein processing and synaptic dysfunction that are defining features of AD.
- In AD, SIRT3 is generally upregulated and it may increase further in response to increased ROS, likely as a protective response. However, where the predisposing APOE4 is present, SIRT3 is downregulated.

(Wilkins HM, Curr Top Med Chem, 2016; Oliver DMA, J Alzheimers Dis, 2019; Desler C, Curr Med Chem, 2018; Gibson GE, Neurochem Res, 2017; Ansari A, Aging Cell, 2017; Fernandez-Moriano C, Oxid Med Cell Longev, 2015)

Alzheimer's disease and mitochondria: further problems with hyperphosphorylated tau protein

- Not only does hyperphosphorylated tau protein form neurofibrillary tangles, but overexpression of tau also inhibits mitochondrial function by decreasing the activity of ETC Complexes (particularly Complexes I and V) leading to reduced ATP synthesis, downregulating antioxidant enzymes and dissipating membrane potential, all leading to reduced synaptic function.
- Abnormal interaction between hyperphosphorylated tau and fission proteins cause excessive mitochondrial fission and lead to the degeneration of mitochondria and synapses in AD patients.
- Hyperphosphorylated tau also impaired the transport of amyloid precursor protein (APP).
- However, some AD mouse models exhibit cognitive impairment before observation of hyperphosphorylated tau and formation of neurofibrillary tangles (NFTs). This raises doubts about whether pathological tau is a trigger of the mitochondrial abnormalities or vice versa. Mitochondrial dysfunction itself can lead to the hyperphosphorylation and aggregation of tau.

(Cheng Y, Front Neurosci, 2018; Manczak M, Hum Mol Genet, 2012; Zhang F, Transl Neurodegen, 2015)

Alzheimer's disease and mitochondria: further problems with amyloid beta (Aβ) and amyloid precursor protein (APP)

- Both APP and Aβ can accumulate in the mitochondria and the extent correlates with mitochondrial dysfunction; early accumulation of Aβ in mitochondria may be an initiating pathological event.
- Aβ is responsible for mitochondrial mutations in AD development and can induce formation of the mtPTP following calcium overload, with loss of membrane potential and release of cytochrome c, leading to mitochondria-mediated apoptosis. Decreased mitochondrial MnSOD expression level has been found in AD patients as well as decreased coenzyme Q10 in neurons.
- It can also stimulate the production of lactate dehydrogenase, which promotes the breakdown of pyruvate into lactate in anaerobic respiration.
- Aβ may be locally produced in mitochondria via mitochondrial gamma-secretase.
- APP can inhibit Complex IV activity and increase ROS production; the higher the level of mitochondrial APP, the worse the mitochondrial dysfunction.

(Kamat PK, Cell Biochem Biophys, 2014; Fernández-Moriano C, Oxid Med Cell Longev, 2015; Akhter F, Prog Mol Biol Transl Sci, 2017; Sheng B, Free Radic Biol Med, 2009; Cheng Y, Front Neurosci, 2018)

Drugs which increase risk of cognitive impairment and dementia

- Anticholinergics: Drugs commonly prescribed for depression, epilepsy, overactive bladder, COPD, psychosis, PD and some allergies, comprising antidepressants, antipsychotics and muscle relaxants. Anticholinergics block the action of acetylcholine, used by the brain to control signalling around the body.
- In 58,000 dementia patients, there was an association between strong anticholinergic medication and increased risk of dementia in those aged >54.
- The adjusted OR for dementia increased from 1.06 in the lowest overall anticholinergic exposure category to in the highest category, compared with no anticholinergic drug prescriptions in the 1 to 11 years before the index date. There were significant increases in dementia risk for the anticholinergic antidepressants (OR 1.29), anti-parkinson drugs (OR 1.52), antipsychotics (OR, 1.70), bladder antimuscarinic drugs (OR 1.65) and antiepileptic drugs (OR 1.39). Associations were stronger in cases diagnosed before the age of 80 years. (Coupland CAC, JAMA Intern Med. 2019)

Drugs for Alzheimer's disease

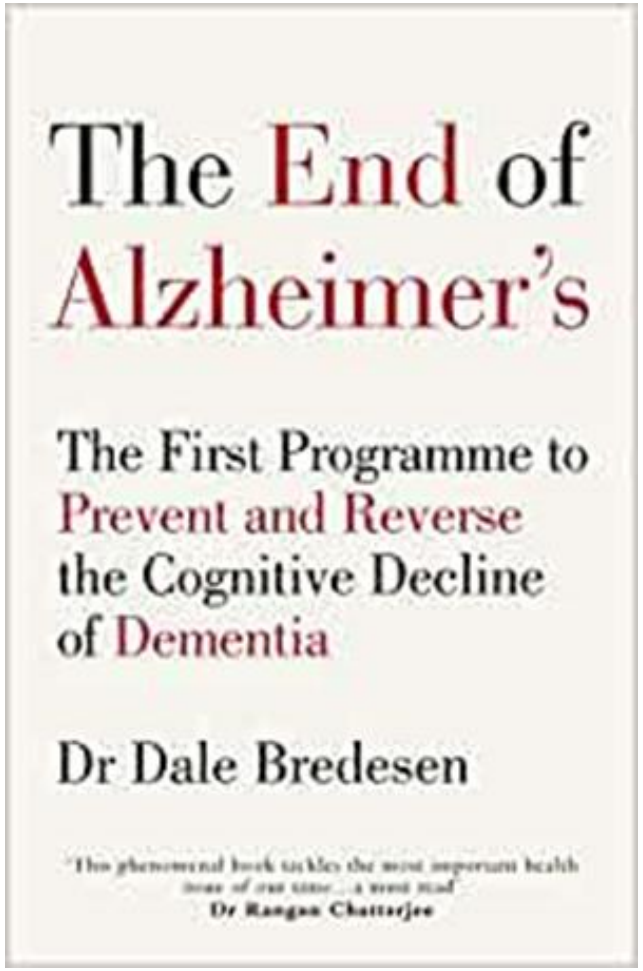
- **Conventional medicine thinks that Aβ accumulation in plaques is the cause of AD, which it calls the 'amyloid cascade' of disease**.
- **Many drugs have been designed to remove or prevent the action of Aβ.** They are mostly antibodies which bind to Aβ and remove it from the brain or block the enzyme which produces Aβ. In these tasks, **they were highly successful**.
- **However, not only did they fail even to stabilise AD, in some cases they significantly increased disease progression. More than 300 drug trials have now been carried out. Virtually none were found to be effective in even slowing AD progression.**
- **Donezepil (Aricept) and Memantine, drugs which can help patients a little, do not act on Aβ.** Aricept is a cholinesterase inhibitor (which retains acetylcholine in the brain for longer), while Memantine reduces the excitotoxicity of glutamate. Neither drug can even slow progression of the disease, let alone reverse it!

In June there was headline news of the US FDA's authorisation of yet another drug: Aduhelm (Aducanumab), an antibody drug.

- However, this is **not a new drug**. Its clinical trials were stopped in 2019 as no improvement at all was seen in AD patients and the drug was **linked to brain oedema**.
- But a re-analysis of the trial data by the manufacturers suggested that **some benefit could be seen if higher doses were given.**
- This is not going to end well!

Dale Bredesen, Professor of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA

- He has **published a number of studies on AD, as well as the highly successful book: The End of Alzheimer's.**
- Bredesen has developed several hypotheses about AD.
- They remain to be independently corroborated in humans but he has managed to cure a number of AD sufferers.



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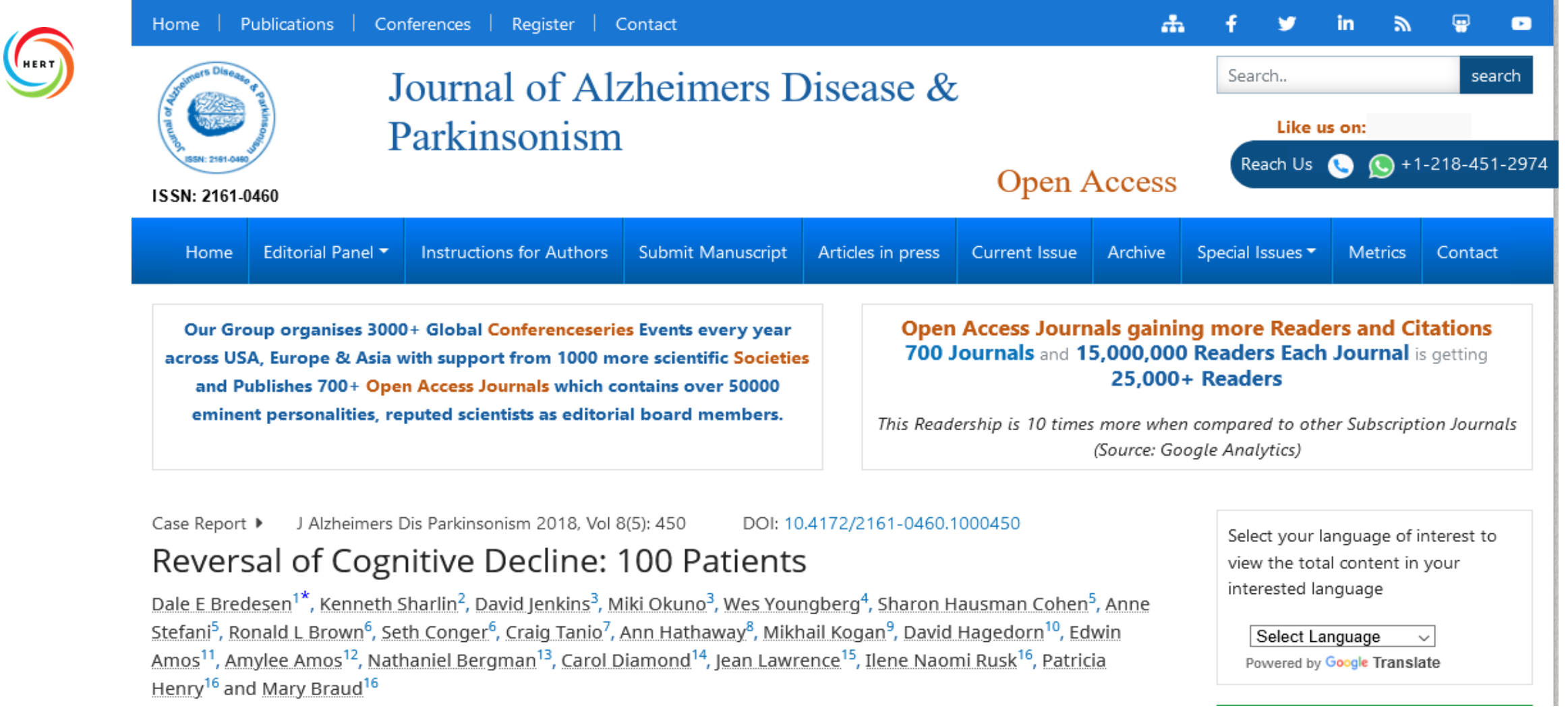
Research Paper

Reversal of cognitive decline in Alzheimer's disease

Dale E. Bredesen[1,2], Edwin C. Amos[3], Jonathan Canick[4], Mary Ackerley[5], Cyrus Raji[6], Milan Fiala[7], and Jamila Ahdidan[8]

Abstract: Alzheimer's disease is one of the most significant healthcare problems nationally and globally. Recently, the first description of the reversal of cognitive decline in patients with early Alzheimer's disease or its precursors, MCI (mild cognitive impairment) and SCI (subjective cognitive impairment), was published [1]. The therapeutic approach used was programmatic and personalized rather than monotherapeutic and invariant, and was dubbed metabolic enhancement for neurodegeneration (MEND). Patients who had had to discontinue work were able to return to work, and those struggling at work were able to improve their performance. The patients, their spouses, and their co-workers all reported clear improvements. Here we report the results from quantitative MRI and neuropsychological testing in ten patients with cognitive decline, nine ApoE4+ (five homozygous and four heterozygous) and one ApoE4-, who were treated with the MEND protocol for 5-24 months. The magnitude of the improvement is unprecedented, providing additional objective evidence that this programmatic approach to cognitive decline is highly effective. These results have far-reaching implications for the treatment of Alzheimer's disease, MCI, and SCI; for personalized programs that may enhance pharmaceutical efficacy; and for personal identification of ApoE genotype.



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Reversal of Cognitive Decline: 100 Patients

Dale E Bredesen[1*], Kenneth Sharlin[2], David Jenkins[3], Miki Okuno[3], Wes Youngberg[4], Sharon Hausman Cohen[5], Anne Stefani[5], Ronald L Brown[6], Seth Conger[6], Craig Tanio[7], Ann Hathaway[8], Mikhail Kogan[9], David Hagedorn[10], Edwin Amos[11], Amylee Amos[12], Nathaniel Bergman[13], Carol Diamond[14], Jean Lawrence[15], Ilene Naomi Rusk[16], Patricia Henry[16] and Mary Braud[16]

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100 AD or pre-AD patients, treated by several different physicians, all using Bredesen's ReCODE protocol, showed documented improvement in cognition, in some cases with improvement in EEG and MRI results as well.

Bredesen's Hypothesis: there are 3 types of AD

- Bredesen found 3 distinct types of AD:
  - **Type 1: driven by systemic inflammation**. Develops in the 60s.
  - **Type 2: driven by insulin resistance, high homocysteine and imbalances in oestradiol, progesterone, testosterone, insulin and vitamin D.** Develops in the 70s.
  - **Type 3: driven by dementogens: toxins, microbes and stress.** Develops from 40s onwards.
- **Types 1 and 2 are strongly influenced by the ApoE4 allele. Type 3 occurs more often in those who are ApoE4 negative and have at least one ApoE3 allele.**
- Type 3 tends to be inhalational, **a manifestation of chronic inflammatory response syndrome (CIRS), due to biotoxins**, including mycotoxins. Sufferers may not present with cognitive impairment (although it will develop later) but will show executive function problems, depression and stress hypersensitivity. No family history. Presents with low serum zinc and zinc/copper ratio, low triglycerides and low ratio of triglycerides to total cholesterol. There may be hormonal abnormalities, particularly related to stress, and the HPA axis may be dysfunctional: low DHEA and morning cortisol.

(Bredesen DE, Aging, 2015; Bredesen DE, Aging, 2016)

Bredeson's ReCODE protocol

- The ReCODE Protocol is not a one-size-fits-all approach and recommends treatment strategies that are **highly specific to individual participants**.
- The first step is to determine **which of the 3 types of AD** or pre-AD the patient has. This will suggest the tests to be run and the potential remedies.
- For Type 1, with proven inflammation: ensure an anti-inflammatory diet with anti-inflammatory supplements.
- For Type 2: test for insulin resistance, high homocysteine, imbalanced sex and thyroid hormones, low vitamin D and treat accordingly with diet and supplements.
- For Type 3: test for toxins and infections and identify other stressors. Eliminate toxins and upregulate detox pathways.
- Some investigations, such as for essential micronutrients will be common to all types. Lifestyle, particularly exercise and sleeping will be optimised and mental therapies such as brain training may also be recommended.

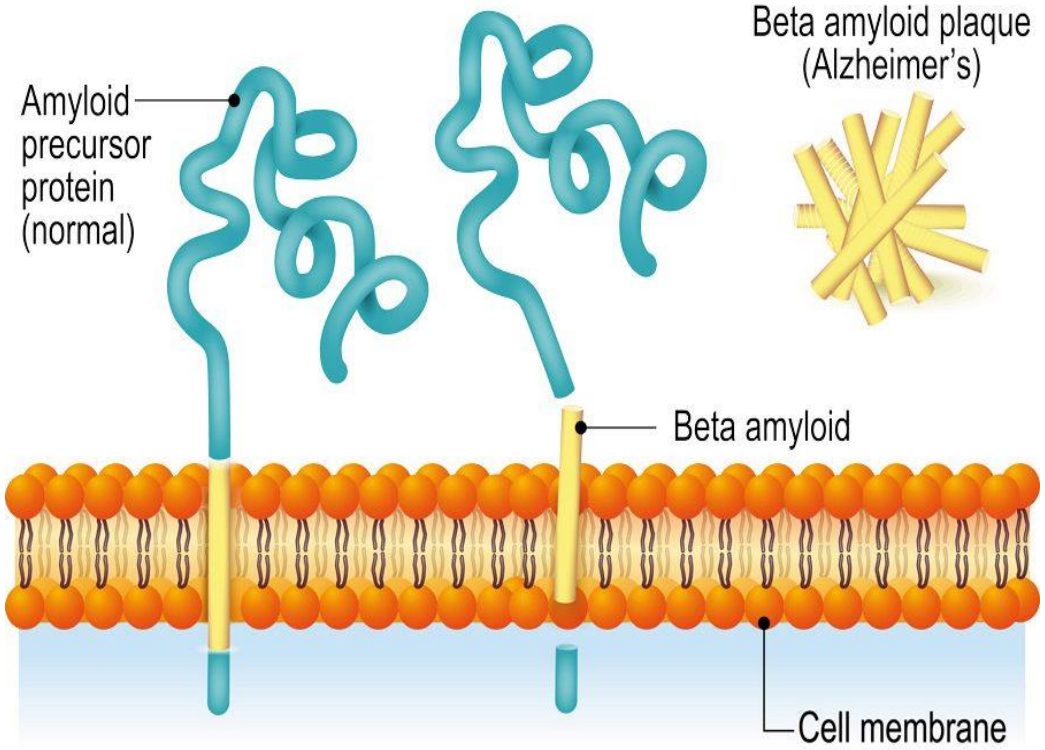
Is there support for Bredesen's clinical approach?

- **Not much, because conventional medicine is still saying that 'there is no cure for Alzheimer's disease** or a way to stop or slow its progression' (The Alzheimer's Association, https://www.alz.org/alzheimers-dementia/treatments).
- However, a recent study of nearly 3000 subject showed that compared to participants with 0 to 1 healthy lifestyle factors, the **risk of Alzheimer dementia was 37% lower in those with 2 to 3 healthy lifestyle factors and 60% lower in those with 4 to 5 healthy lifestyle factors**. Healthy lifestyle factors comprised:
  - non-smoking,
  - ≥150 min/wk moderate/vigorous-intensity physical activity,
  - light to moderate alcohol consumption,
  - high-quality Mediterranean-DASH Diet,
  - engagement in late-life cognitive activities.

(Dhana K. Neurology Jul 2020, 95(4): e374-e383)

The amyloid cascade hypothesis: Bredesen's views

- Bredesen has shown that **Aβ is present in the brain and CSF of normal healthy individuals.**
- Furthermore, post mortem studies have found **brains riddled with amyloid plaques, but in people who had died in their nineties and had retained an excellent memory** up until death.
- So he starts from the premise that **Aβ is not necessarily neurotoxic.**



Bredesen hypothesis on the true role of amyloid β

- Bredesen proposes that amyloid precursor protein (APP) **produces amyloid as a protective response whenever a threat is detected in the brain**.
- These threats could take several forms:
  - Inflammation (from toxins, infection, diet, lifestyle)
  - Oxidative stress (from toxins, infection, diet, lifestyle)
  - Shortage of nutrients (poor diet, nutrient-poor foods)
  - Insulin resistance (high sugar and carb intake, toxins)
- **He believes that amyloid is part of the innate immune system, is antimicrobial and can bind some toxins**, such as mercury and divalent metals such as iron.
- **This is why patients get worse if Aβ is removed with drugs. The microbes and toxins are still in the brain but there is no amyloid to neutralise them.**

So where is the evidence that Aβ is protective?

- In vitro studies show that Aβ 1-42 in monomer form protects developing and mature neurons against excitotoxic death under conditions of trophic deprivation (Giuffrida ML, J Neurosci, 2009). Aβ 1-42 carries out its protective role via insulin receptor signalling – another reason why insulin resistance could induce pathology.
- In mice with induced MS, treatment with Aβ peptides reduced motor paralysis and brain inflammation. Importantly, the protection conferred by Aβ treatment did not require its delivery to the brain; instead, treated lymphocytes were injected in the body. (Grant JL, Sci Transl Med, 2012)
- APP-deficient zebrafish had fewer cerebrovascular branches and shorter vessels, which was rescued by treatment with human Aβ peptide. There was a direct correlation between extracellular levels of Aβ and cerebrovascular density in the developing hindbrain. (Luna S, PLOS One, 2013)
- A 1997 study concluded that 'Aβ fragments…attenuate the toxicity of aluminium' (van Rensburg SJ, S Afr Med J, 1997)
- A review article discusses the evidence that Aβ is not the cause of AD but a protective device against it (Lee HG, J Pharmacol Exp Ther. 2007).

And the evidence that Aβ is antimicrobial? SS

- Several studies have shown that viral, bacterial, mould or parasitic infections are prevalent in AD patients (Kountouras J, Int J Neurosci, 2009; Kountouras J, J Neurol, 2009; Readhead B, Neuron, 2018; Balin BJ, J Alz Dis. 2008; Honjo K, Alzheimers Dement. 2009; Tzeng NS, Neurotherapeutics, 2018).
- A 2020 News Feature in Nature made the point that for years this was a 'fringe theory' but now researchers are taking it seriously (https://www.nature.com/articles/d41586-020-03084-9). It has given rise to the 'Antimicrobial Protection Hypothesis' and there are suggestions that Aβ might be developed as a natural antibiotic. One paper noted that the antimicrobial role of Aβ may explain why increased rates of infection have been observed in some of the AD clinical trials that depleted Aβ. (Moir RD, Alzheimers Dement. 2018; Gosztyla ML, J Alzheimers Dis. 2018).
- Aβ exerts antimicrobial activity against eight common and clinically relevant microorganisms; this effect was abolished with anti-Aβ antibodies (Soscia SJ, PLoS One, 2010). One team injected HSV1 into the brains of young transgenic mice; within three weeks, their brains were dotted with amyloid plaques. These amyloid plaques proved to be life-saving; when the team gave a lethal dose of HSV1, amyloid plaques appeared in the brain of the transgenic mice within 2 days and they lived significantly longer than the controls. (Eimer WA, Neuron, 2018)
- Aβ expression protects against fungal and bacterial infections in mouse, nematode, and cell culture models of AD. Aβ acts by disrupting microbial membranes to capture, agglutinate and finally entrap microbes in a network of β-amyloid, which is then marked (opsonised) for phagocytosis (Kumar DKV, Sci Transl Med, 2016).
- Aβ can also be induced by LPS, endotoxins released by gram-negative bacteria (Wang LM, Am J Nucl Med Mol Imaging. 2018).

AD from microbes in the brain

- First proposed in 1991 but ridiculed by conventional medicine (Jamieson GA, Latent herpes simplex virus type 1 in normal and Alzheimer's disease brains, J Med Virol, 1991)
- This prompted entrepreneur Leslie Norins to offer US$1 million of his own money to any scientist who could prove that Alzheimer's disease was caused by a germ. The hypothesis suggests that instead of just being a toxic waste product, amyloid might help to protect the brain from infection.
- Critics of the amyloid hypothesis note that the brains of many people who did not have Alzheimer's disease have been shown to contain plaques on post-mortem. And they point to the failure of many clinical trials of treatments designed to dissolve amyloid plaques, none of which has slowed the disease.
- Many researchers have found brain microbes in post mortem studies but this does not prove causation. Nevertheless, several microbes have been proposed as triggers of Alzheimer's, including human herpes viruses, Chlamydia pneumoniae, Borrelia burgdorferi, and Porphyromonas gingivalis. (Readhead B, Neuron. 2018;)
- In theory, any infectious agent that can invade the brain could have this trigger role (there's no good evidence, however, that SARS-CoV-2, the virus behind COVID-19, has this ability).
- Certainly AD seems to be more prevalent with certain infections; researchers found a hazard ratio of >2.5 for the development of dementia in those with herpes simplex virus (Tzeng NS, Neurotherapeutics, 2018)
- Nevertheless, one team found that Abeta exerts antimicrobial activity against eight microorganisms (Soscia SJ, PLoS One, 2010). They went on to inject HSV1 into the brains of young transgenic mice; within three weeks, the brains of the transgenic mice were dotted with amyloid plaques. These amyloid plaques proved to be life-saving; when the team gave a lethal dose of HSV1, amyloid plaques appeared in the brain of the transgenic mice within 2 days and they lived significantly longer than the controls (Eimer WA, Neuron, 2018)
- (https://www.nature.com/articles/d41586-020-03084-9)

So if Aβ is so protective, why do neurons die?

- Because Aβ is not the only factor needed. We also need neurotrophic factors (proteins that induce the survival, development and function of neurons). These include nerve growth factor, brain derived neurotrophic factor (BDNF), neurotrophins, etc.
- A neuron or synapse that cannot be adequately maintained is better dead. So the decision is made that they must be sacrificed so that scarce resources (neurotrophic factors) can focus on those that have a chance of survival.
- APP therefore triggers caspases to trigger apoptosis in neurons and synapses to a level at which they can be adequately supported by the scarce neurotrophins. APP balances the threats and availability of neurotrophins to determine whether there is adequate support for neurons and synapses.
- Bredesen says Alzheimer's is essentially a "scorched-earth retreat" so that the microbe or toxin cannot take advantage of the situation.

Dr Stephanie Seneff on cholesterol in the brain

- Studies have shown that although only 2% of the body's mass, the **brain should contain 25-30% of the body's cholesterol, mostly in the form of myelin, comprised of saturated fat, cholesterol, omega 3 fats and a few omega 6 fats.** Cholesterol plays a critical role both in nerve transport in the synapse and in encouraging growth of neurons through healthy development of the myelin sheath coating nerve fibres, which is comprised of fatty acids and cholesterol; without these components, ataxia and tremor occurs. (Saher G, Nat Neurosci, 2005)
- **Furthermore, cholesterol is the limiting factor in the growth of synapses.**
- **Cholesterol can also protect the brain from invasive bacteria and viruses**; it is the first line of defence against microbes and signals white blood cells to attack. It has also been found to be low in those who died from trauma or sepsis, **leading researchers to consider therapies to raise cholesterol levels in the brain** (Wilson RF, Critical Care, 2003).
- In fact, **high serum cholesterol is positively correlated with longevity in people aged >85**, where each 1 mmol/L increase in total cholesterol corresponded to a 15% decrease in mortality (Weverling-Rijnsburger AWE, Lancet, 1997).
- Similarly, those with LDL levels <70 mg/dL had a significantly higher risk of developing intracerebral haemorrhage compared to those with higher levels (Ma C, Neurology. 2019).
- Cholesterol is so important in the brain, that it can be synthesised by astrocytes, not just in the liver (Pfrieger FW, BioEssays, 2003).

(Seneff S, Eur J Intern Med. 2010; 22(2):134-40; http://people.csail.mit.edu/seneff/alzheimers_statins.html)

Dr Stephanie Seneff on cholesterol and AD

- **High serum cholesterol** was associated with **improved memory** in those without the APO E4 allele (West R, Am J Geriatr Psychiatry, 2008) and with **reduced dementia**, but the risk reduction was apparent only among those **with the highest serum levels** (Mielke MM, Neurology, 2005).
- **AD post-mortem studies** have found **poor quality myelin sheaths** and **severe deficiency of fatty acids in the cerebral spinal fluid (CSF)** (as little as 1/6th of levels in healthy controls), phospholipids and cholesterol, which is unrelated to ApoE4. **The difference in fat content was startling: 28 µmol/L in non-AD brains, 4.5 µmol/L in AD brains.** (Mulder M, Alzheimer Dis Assoc Disord. 1998).
- **Seneff asks if the low fat craze could have been partly responsible for the increase in neurodegenerative disease and whether high cholesterol could be a protective mechanism against Alzheimer's?**
- Similarly, lower CSF cholesterol, phospholipids and fatty acids are associated with higher incidence of AD (Notkola IL, Neuroepidemiology, 1998). Of course, this will have relevance for multiple sclerosis as well.
- In addition, the Shanghai Aging Study found that high LDL cholesterol was inversely associated with dementia (i.e. it appeared to be protective) (Zhou F, Front Neurol, 2018). These findings were echoed in a Korean study (Kim JM, J Nutr Health Aging. 2002), while a US study showed that higher levels of LDL and total cholesterol were risk factors for dementia or AD (Reitz C, Arch Neurol, 2004).

(Seneff S, Eur J Intern Med. 2010; 22(2):134-40; http://people.csail.mit.edu/seneff/alzheimers_statins.html)

Dr Stephanie Seneff on statins

- **Despite many studies investigating whether high cholesterol is a risk factor for AD, only 1 found a correlation**, among males in their 50s, who were more likely to develop AD later in life (Anstey KJ, Am J Geriatr Psychiatry, 2008). **Nevertheless, Big Pharma have seized on this study to promote statins to prevent AD, even though the vast majority of studies show no correlation.**
- **In fact, a 2016 Cochrane Review of 2 large population-based studies found that statins are not protective at all;** there were no differences between statin and non-statin groups at risk of vascular disease on five different cognitive tests (McGuinness B, Cochrane Database Syst Rev. 2016).
- And a more recent study showed that **statins more than doubled the risk of dementia in patients with mild cognitive impairment**, with PET scans showing a substantial decline in metabolism in the posterior cingulate cortex, the region of the brain known to decline the most significantly in the earliest stages of Alzheimer's disease (Padmanabham P, J Nuclear Med, 2021).
- **Seneff therefore concluded that statins increase susceptibility to Alzheimer's, partly through lowering natural healthy levels of cholesterol in the brain.** **
- Indeed, a common side-effect of statins is memory and cognitive dysfunction (Timimi F, J Med Internet Res, 2019). Although in some individuals statins may be preventive against dementia, in others they induce cognitive impairment (Schultz BG, Transl Neurodegener. 2018)
- But statins lower much-needed cholesterol, reduce supply of fatty acids and antioxidants to the brain (because they reduce the number of LDL particles, which carry the fatty acids and antioxidants), inhibit production of CoQ10 (since it has the same metabolic pathway as cholesterol) and reduce vitamin D production from UV rays from sunlight (since this requires cholesterol). Others have argued that dementia is both directly and indirectly associated with a vitamin D deficiency.

Vascular dementia and dementia with Lewy bodies also involve mitochondrial dysfunction

- Vascular dementia (VD) is considered to be the second most common cause of cognitive impairment after Alzheimer's disease in the elderly. VD is a clinical syndrome that encompasses a wide spectrum of cognitive disorders caused by cerebrovascular disease. Increasing evidence shows that inflammation and mitochondrial damage are two important mechanisms of VD after stroke. Mitochondrial dysfunction principally occurs as a result of hypoxia. (Sun L, Exp Ther Med, 2018)
- Dementia with Lewy bodies (DLB) is characterised by attentional impairment, visual hallucinations, extrapyramidal features, a fluctuating course, REM-sleep disorder and neuroleptic hypersensitivity. The presence of Lewy bodies leads to significant impairment of nigrostriatal dopaminergic and basocortical cholinergic neurotransmission. However, the chronic microglial activation and neuroinflammation seen is AD is absent in DLB.
- DLB is caused by an abnormal aggregation of the fibrillar form of alpha-synuclein in the neurons. Both dopamine and Aβ1-42 may promote alpha-synuclein accumulation and neurodegeneration. Mitochondria play an important role in the formation of Lewy bodies through increased sensitivity and/or apoptosis through the release of cytochrome c. The mitochondrial mechanisms involved in DLB can involve either mutations of alpha-synuclein, which causes mitochondrial damage, or dysfunction of mitochondrial energy metabolism in neurons. (Spano M, Funct Neurol, 2015; Rajkumar AP, Am J Geriatr Psychiatry, 2020).

Treatment of AD, dementia and mild cognitive impairment (MCI) with mitochondrial therapies (1)

- Caloric restriction or fasting: No human studies. In animals CR improves neuronal energy production and neurotransmission rates and protects against excitotoxicity, neuronal apoptosis and oxidative stress (Gültekin F, Metab Brain Dis, 2018; Amigo I, Aging Cell, 2017; Lin AL, J Cereb Blood Flow Metab, 2014).
- Ketogenic diet: An MCT-based ketogenic formula improved verbal memory and processing speed in patients with AD (Ota M, Neurosci Lett, 2019), while in subjects with MCI, ketone levels were positively correlated with memory performance (Krikorian R, Neurobiol Aging, 2012). Compared with a low-fat diet, patients on the ketogenic diet improved in daily function and quality of life (Phillips MCL, Alz Res Therapy, 2021). Higher ketone values were associated with greater improvement in paragraph recall and increased the stability of brain networks (Reger MA, Neurobiol Aging. 2004; Mujica-Parodi LR, PNAS, 2020). A 2020 review showed that in animals, the ketogenic diet induced an improvement in cognition and motor function in AD mouse models and ageing animals (Lilamand M, Alzheimers Res Ther, 2020), while a review of human and animal studies suggests that ketones may be beneficial (Henderson ST. Neurotherapeutics. 2008). Ketones can protect neurons against dysfunction and degeneration in animal models of AD (Mattson MP, Ageing Res Rev, 2015).
- Exercise: A 2018 systematic review showed that aerobic exercise had positive effects on patients' global cognition (Cammisuli DM, Arch Ital Biol, 2018). Exercise can also delay the decline in cognitive function in AD patients (Panza GA, J Am Geriatr Soc, 2018). Although all exercise can help, aerobic exercise raises BDNF levels in dementia patients (Liu IT, Arch Phys Med Rehabil, 2020).

Treatment of AD, dementia and mild cognitive impairment (MCI) with mitochondrial therapies (2)

- Hyperbaric oxygen: In patients with AD or MCI, hyperbaric oxygen (HBO) therapy for 40 mins/day for 20 days significantly improved cognitive function and improved brain glucose metabolism (Chen J, Alzheimers Dement, 2020). In patients with vascular dementia, HBO for 60 mins/day, 5 days/week for 12 weeks also improved cognitive function (Xu Y, Cell Transplant, 2019).
- Hypothermia: Lower body temperature and ageing increases tau hyperphosphorylation but in a mouse model of AD, repeated cold exposure protects against cold-induced tau phosphorylation (Tournissac M, Mol Metab, 2019).
- Near infra-red radiation: A 2017 study found that a significant improvement in cognitive and behavioural function, sleep, angry outbursts, anxiety and wandering after 12 weeks of photobiomodulation; it also improved rule-based learning and cognitive function (Saltmarche AE, J Photomed Laser Surgery, 2017; Chao LL, Photobiomodul Photomed Laser Surg. 2019; Blanco N, J Neuropsychol, 2017; Vargas E, Lasers Med Sci, 2017). In healthy young adults, transcranial infrared laser stimulation and acute aerobic exercise treatments were similarly effective for cognitive enhancement (Hwang J, Lasers Med Sci, 2016). In vitro, it upregulates BDNF and rescues neurons loss and dendritic atrophy (Meng C, 2013, J Neurosci). In rodent studies, infrared reduces cognitive decline, β-amyloid protein levels, hyperphosphorylated tau, neurofibrillary tangles and oxidative stress markers in the AD brain (Grillo SL, J Photochem Photobiol B, 2013; Purushothuman S, Alzheimers Res Ther, 2014; Wang M, Biochem Biophys Res Commun, 2020; Hamblin MR, BBA Clinical 2016).
- Pulsed electromagnetic fields: No human studies. In in an experimental cell model of AD, PEMFs modulated gene expression in cell functions that are dysregulated in AD (Capelli E, J Healthc Eng, 2017). In rats with T2D-induced dementia, PEMFs improved learning and memory (Li Y, Electromagn Biol Med, 2019).

Mitochondrial remedies for which there is evidence for Alzheimer's disease, dementia or mild cognitive impairment (Annex D)

- Astaxanthin
- B vitamins
- Berberine
- Butyrate
- Cannabinoids
- Capsaicin
- L-carnitine
- Coenzyme Q10
- Creatine
- Ginkgo biloba
- Ginseng
- Supplementary ketones and MCTs
- Lipoic acid
- Magnesium
- Melatonin
- N-acetyl cysteine
- NAD+ and precursors
- Omega 3 fatty acids
- Selenium *
- Taurine
- Vitamin A *
- Vitamin D

Flavonoids and isoflavones

- Baicalin/Baicalein
- Curcumin
- Epigallocatechin-3-gallate (EGCG)
- Icariin
- Luteolin
- Naringin/Naringenin
- Nobiletin
- Quercetin
- Resveratrol
- Genistein
- Puerarin

Vitamins and minerals with *

All essential for mitochondrial function but in excess can be highly toxic.

Both deficiency and excess induce mitochondrial damage.

Flavonoids

A combination works best, as this is how they arise in nature. Their individual effects complement each other.

Top 5 remedies for Alzheimer's disease, dementia or mild cognitive impairment in humans

- Acetyl L-carnitine: 1500mg-4000 mg/day
- Panax ginseng: 3000mg-4500mg/day
- MCT oil: 17.3g/day
- Alpha lipoic acid: 600mg/day
- Vitamin D: 800-2000IU/day – do not give with calcium

Parkinson's disease (PD) and Parkinsonism

- Parkinson’s disease is the 2nd most common neurodegenerative disorder and the most common movement pathology, affecting about 1% of the population over the age of 60. Typical PD motor symptoms are muscle rigidity, tremor and freezing of gait.
- PD is characterised by progressive neurodegeneration and neuronal loss in many brain areas, but particularly among the dopaminergic neurons of the *substantia nigra* (SN), along with the formation of Lewy bodies (abnormal aggregations of proteins, principally α-synuclein, that develop inside neurons). Altered protein handling, particularly of the ubiquitin-proteasome system, is thought to play a key role in the pathogenesis of PD.
- There are 2 types of PD: familial and idiopathic (sporadic)
    1. Familial forms of PD are characterised by mutations of genes encoding proteins associated with sensing of oxidative stress or the mitochondrial quality control system, such as PINK1, Parkin and α-synuclein. Mutations in Parkin and PINK1 genes are known to cause autosomal recessive forms of PD and have been implicated in the control of mitochondrial morphology and function. Familial PD accounts for about 10% of all cases.
    2. Idiopathic (sporadic) PD (around 90% of cases) can be caused by environmental factors (despite the term ‘idiopathic’), such as pesticides. The pesticide Rotenone, a Complex I inhibitor, produces widespread neuronal death and is typically used to induce PD in animal models.
- Point mutations in the α-synuclein gene can cause familial forms of PD, while single nucleotide polymorphisms (SNPs) are associated with an increased risk of developing sporadic PD.
- In PD patients, dopamine is low in part because of reduced activity of tyrosine hydroxylase, often die to low levels of the cofactor for tyrosine hydroxylase, tetrahydrobiopterin; both require NADH, which has been shown to increase dopamine production 6-fold in nerve cell culture.

(Olanow CW, Mov Disord, 2013; McNaught KS, Olanow, 2001; Olanow CW, Perl, 2004; Kamat PK, Cell Biochem Biophys, 2014; Nissanka N, FEBS Letts, 2018; Valdinocci D, Front Neurosci, 2019; Nissanka N, FEBS Letts, 2018; Kamat PK, Cell Biochem Biophys, 2014; Bondi H, J Neurochem, 2016; Burchell VS, Expert Opin Ther Targets, 2010; Duchen MR, Eur J Physiol, 2012; Vrecko K, J Neural Transm, 1993; Birkmayer JGD, Acta Neurol Scand, 1989; Birkmayer JGD, Acta Neurol Scand, 1993)

Mitochondrial involvement in PD and Parkinsonism

- Mitochondrial dysfunction is strongly implicated in the pathogenesis of both familial and idiopathic PD: reduced mitochondrial potential, ETC Complex inhibition (mainly Complex I), lower ATP production, iron accumulation and oxidative stress in dopaminergic neurons of the substantia nigra and downregulation of the genes for mitochondrial PINK1 and Parkin production.
- Decreased PINK1 and Parkin can lower mitophagy, despite high levels of mitochondrial fission, leaving damaged and dysfunctional mitochondria in the cell. Surviving dopaminergic neurons have high levels of mtDNA deletions; 40–60% of mtDNA deletions were associated with cytochrome c oxidase deficiency.
- Dopaminergic neurons in the *substantia nigra* are uniquely vulnerable to oxidative damage, having a high content of oxidisable dopamine, neuromelanin, PUFAs and metals, particularly iron, and relatively low antioxidant availability with a high metabolic rate. There may also be increased intracellular calcium excitotoxicity and protein misfolding and aggregation.
- Post mortem studies have shown dysfunctional and uncoupled mitochondria and decreased autophagy.
- Animal models involving toxic damage to the CNS using mitochondrial Complex I inhibitors all promote PD-like symptoms and formation of α-synuclein-containing protein aggregates and cause mitochondrial and other damage to dopaminergic neurons; PGC-1α inhibition can also promote sporadic PD.
- It is thought that PD pathology spreads between adjacent brain regions by lateral (mitochondrial) gene transfer.

(Kidd PM, Altern Med Rev, 2000; Burchell VS, Expert Opin Ther Targets, 2010; Chen C, Biology, 2019; Videira PAQ, Front Neurosci, 2018; Nissanka N, FEBS Letts, 2018; Duchen MR, Eur J Physiol, 2012; Jiang X, Oxid Med Cell Longev, 2019; Bondi H, J Neurochem, 2016; George S, J Mol Neurosci, 2010; Aguirre P, Biometals, 2012; Kamat PK, Cell Biochem Biophys, 2014; Scherer TB, J Neurosci, 2002; Esteves AR, Biochim Biophys Acta, 2014; Berndt N, Int J Cell Biol; Alberio T, Biochim Biophys Acta, 2014; Feng ST, Pharmacol Res, 2019; Valdinocci D, Front Neurosci, 2019; Jiang X, Oxid Med Cell Longev, 2019; Fernandez-Moriano C, Oxid Med Cell Longev, 2015)

Key features of mitochondria and Parkinson's disease (PD)

- α-synuclein is healthy neurons has many beneficial functions but in dopaminergic neurons in PD it aggregates, misfolds, forms insoluble fibrils, inhibits Complex 1 and induces mitochondrial dysfunction. It is an important protein in MAM function, and its misfolding has given rise to the 'MAM-hypothesis' of PD; it may also be a prion protein.
- After the administration of L-Dopa, rat studies have shown a dose-dependent reduction in ATP production, as well as increased superoxide radical formation and oxidative damage. L-Dopa can initiate protein misfolding through its ability to mimic L-tyrosine, which leads to mitochondrial dysfunction.
- Mutations in PINK1 and Parkin genes, which lead to defects in mitochondrial quality control, can result in early-onset autosomal recessive PD. Similarly, downregulation of mitochondrial PINK1 and Parkin production reduces mitophagy via imbalanced mitochondrial fusion and fission and leads to cell death.
- There is commonly a defect in synthesis of iron-sulphur clusters (ISC) in PD, which induces partial inhibition of Complex I, which itself can mimic PD.
- Calcium-induced cell injury has been implicated in the pathogenesis of PD, making mitochondria vulnerable to calcium overload and reducing the effective threshold for mtPTP opening, leading to calcium-dependent cell death. Intracellular calcium can also interact with a-synuclein to promote its aggregation.
- Several researchers are now investigating whether PD may in part be an autoimmune disease, and that signs of autoimmunity can appear in Parkinson's disease patients years before their official diagnosis. Peptides derived from α-synuclein can act as antigenic epitopes and drive T cell responses and antibody production in PD patients. (Sulzer D, Nature, 2017; Garretti F, Front Immunol. 2019; Arlehamn CSL, Nat Commun, 2020; Yanamandra K, PLoS One, 2011; Campos-Acuna J, Front Immunol, 2019)

α-synuclein and mitochondria in PD

- In healthy neurons, α-synuclein is found mainly in pre-synaptic terminals, where it thought to regulate neurotransmitter release, protects against apoptosis and aid in DNA repair and mobilisation of the innate immune response against pathogens. But when it forms protein aggregates and insoluble fibrils, as found in Lewy bodies and Lewy neurites, its presence becomes pathological and it becomes the principal histopathological marker of PD in dopaminergic neurons; α-synuclein pathology is also seen in AD.
- Although the reason for α-synuclein aggregation is not fully understood, it appears to be triggered by raised copper concentrations, increased calcium binding or oxidative stress.
- It is unclear whether the presence of α-synuclein in mitochondrial membranes is itself pathological or merely an indicator of stress conditions. It is known to be an important protein in MAM function but its misfolding in dopaminergic neurons is a characteristic of PD and has given rise to the 'MAM-hypothesis' of PD. Impairment of the MAM can lead to poor calcium transfer, resulting in mitochondrial fragmentation.
- α-synuclein can also lead to Complex 1 inhibition and mitochondrial dysfunction, found principally in dopaminergic neurons but also in platelets and lymphocytes. α-synuclein can bind to lipids in the outer mitochondrial membrane, altering the membrane curvature and leading to a decrease in mitochondrial fusion rate relative to fission, producing smaller, less elongated and more fragmented mitochondria which are unable to travel down the neuronal cytoskeletal tracks to synapses, impacting neuronal signalling.
- α-synuclein may also be a prion-like protein, making PD a prion-like disease. Studies suggest that misfolded α-synuclein can migrate to unaffected neurons, where it can act as a template to promote misfolding of host α-synuclein. This leads to the formation of larger aggregates, neuronal dysfunction and degeneration and motor disturbances.

(Chen C, Biology, 2019; Emanzadeh FN, J Res Med Sci, 2016; Valdinocci D, Front Neurosci, 2019; Batlevi Y, Neurobiol Dis, 2011; Burchell VS, Expert Opin Ther Targets, 2010; Cali T, J Biol Chem, 2012; Guardia-Laguarta C, Mov Disord, 2015; Olanow CW, Mov Disord, 2013; Raturi A, Biochim Biophys Acta, 2013; Schon EA, J Alzheimer's Dis, 2010; Tubbs E, Diabetes, 2014; Chaudhari N, Front Cell Neurosci, 2014)

L-dopa and mitochondria in PD

- L-dopa, the dopamine precursor, is commonly prescribed for symptom alleviation, although it only works on a temporary basis and does not improve underlying disease pathology.
- After the administration of L-Dopa, rat studies have shown a dose-dependent reduction in ATP production, as well as increased superoxide radical formation and oxidative damage.
- Furthermore, L-Dopa can initiate protein misfolding through its ability to mimic L-tyrosine. This leads to mitochondrial dysfunction and upregulation in the endosomal-lysosomal degradation system.
- These results are counter-intuitive and suggest that artificially increasing the amount of a deficient substance is not always the answer.

(Kidd PM, Altern Med Rev, 2000; Burchell VS, Expert Opin Ther Targets, 2010; Olanow CW, Mov Disord, 2018; Giannopoulos S, Int J Biochem Cell Biol, 2019; Lowes H, Mol Neurodegener, 2020)

Mitochondrial PINK1 and Parkin in PD

- The principal roles of PINK1, a mitochondrial serine/threonine kinase, and Parkin, a cytoplasmic ubiquitin ligase, in healthy cells appear to be mitochondrial quality control; they are thought to protect cells from stress-induced mitochondrial dysfunction. To achieve this, PINK 1 activates Parkin.
- PINK1 is normally imported into the inner mitochondrial membrane but when a mitochondrion becomes uncoupled, protein import is prevented so PINK1 accumulates on the outer mitochondrial membrane, where it monitors the mitochondrion for damage. When damage is detected, it recruits Parkin from the cytosol to bind to depolarised mitochondria to induce mitophagy and aid cell survival.
- Mutations in PINK1 and Parkin genes, which lead to defects in mitochondrial quality control, can result in early-onset autosomal recessive PD.
- Similarly, downregulation of mitochondrial PINK1 and Parkin production reduces mitophagy via imbalanced mitochondrial fusion and fission and leads to cell death.

(Youle RJ, Science, 2012; Kamat PK, Cell Biochem Biophys, 2014; Batlevi Y, Neurobiol Dis, 2011; Bondi H, J Neurochem, 2016; Burchell VS, Expert Opin Ther Targets, 2010; Dagda RK, J Bioenerg Biomembr, 2009; Lazarou M, J Cell Biol, 2013)

Iron and iron-sulphur cluster (ISC) involvement in PD pathogenesis

- A significant body of data supports a role for a defect in synthesis of iron-sulphur clusters (ISC) in PD; iron-sulphur clusters aid electron transport in the ETC. Reduction in ISC synthesis induces partial inhibition of Complex I of the ETC (which includes several ISC-containing subunits), which can mimic PD.
- Reduced ISCs also results in lowered OXPHOS and iron accumulation in *substantia nigra* dopaminergic neurons of PD patients; iron-induced oxidative damage is implicated in age-dependent neuronal loss.
- Furthermore, loss of activity of mitochondrial aconitase, a TCA cycle enzyme that requires iron-sulphur clusters for function and stability, is a marker of ageing. Iron chelation protects *substantia nigra* neurons in PD patients.
- Studies reveal that Friedreich ataxia (FRDA) , PD and brain ageing share certain common aspects, which involve iron accumulation in specific regions of the central and/or peripheral nervous systems, leading to iron-catalysed Fenton chemistry, together with iron-sulphur enzyme deficits. In spite of these three conditions being clinically, pathologically and aetiologically very different, this suggests a common underlying mechanism involving iron metabolism, likely through defects in the biogenesis of mitochondrial iron-sulphur clusters (ISCs).
- This all suggests that the possibility of iron toxicity in PD should receive a lot more attention!

(Isaya G, Front Pharmacol, 2014; Franco-Iborra S, Front Neurosci, 2018; Park JS, Neurochem Int, 2018)

Intracellular calcium and mitochondria in PD

- Calcium-induced cell injury due to delayed mitochondrial efflux has been implicated in the pathogenesis of PD. The functional consequence of this is to make mitochondria far more vulnerable to calcium overload and to reduce the effective threshold for mtPTP opening, leading to calcium-dependent cell death. In PD pathogenesis, this has been seen particularly in dopaminergic neurons of the *substantia nigra*, probably due to their intrinsic susceptibility.
- Intracellular calcium can also interact with a-synuclein to promote its aggregation.
- L-type calcium channel blockers such as dihydropyridine have been shown to protect against PD toxins in animal models.

(Duchen MR, Eur J Physiol, 2012; Valdinocci D, Front Neurosci, 2019; Ammal Kaidery N, Neurochem Int, 2018; Burchell VS, Expert Opin Ther Targets, 2010)

Parkinson’s disease may be an autoimmune condition

- The team led by Alessandro Sette and David Sulzer at La Jolla Institute for Immunology have published some interesting studies showing that Parkinson's disease may be partly an autoimmune disease and that signs of autoimmunity can appear in Parkinson's disease patients years before their official diagnosis.
- Evidence from a number of studies has shown the involvement of the adaptive immune system in Parkinson's disease and specifically the major histocompatibility complex (MHC), involved in antigen presentation to T cells.
- Peptides that are derived from α-synuclein act as antigenic epitopes displayed by MJCs and drive helper and cytotoxic T cell responses in patients with Parkinson's disease. (Sulzer D, Nature, 2017)
- PD patients show higher numbers of T cells in the ventral midbrain than healthy, age-matched controls; these T cells can generate an autoimmune response to α-synuclein, leading to altered protein processing and possibly the generation of neo-epitopes (self-peptides) that have not been identified by the host immune system as ‘self’. Genome-wide association studies have shown associations of PD with haplotypes of major histocompatibility complex (MHC) class II genes, and a polymorphism in a non-coding region that may increase MHC class II in PD patients. (Garretti F, Front Immunol. 2019)
- A longitudinal case study revealed that elevated α-syn-specific T cell responses were detected prior to the diagnosis of motor PD and were highest shortly after diagnosis of motor PD and then decreased; few patients still have them ten years after diagnosis. These results confirm the presence of α-syn-reactive T cells in PD and show that they are most abundant immediately after diagnosis of motor PD. These cells may be present years before the diagnosis of motor PD, suggesting avenues of investigation into PD pathogenesis and potential early diagnosis. (Arlehamn CSL, Nat Commun, 2020)
- Similar associations have also been observed in Alzheimer’s disease (Arlehamn CSL, Curr Opin Immunol).
- Others have found α-synuclein reactive antibodies in blood of Parkinson's disease patients, as well as T cell-driven inflammation in the gut of PD patients (Yanamandra K, PLoS One, 2011; Campos-Acuna J, Front Immunol, 2019).

Mitochondrial therapies in PD

- Fasting and caloric restriction (CR): No human studies. CR protects against degeneration of dopamine neurons and alleviates α-synuclein toxicity. It also increases neurotrophic factors, increasing locomotor activity in animals by upregulating ghrelin/AMPK signalling (Coppens J, Int J Mol Sci, 2017; Guedes A, Mech Ageing Dev, 2017; Bayliss JA, J Neurosci, 2016).
- Ketogenic diet: In the single human study, the ketogenic diet was superior to a low fat diet in improving symptom scores. In rats, a ketogenic diet improved motor function and protected dopaminergic neurons against neurotoxicity by upregulating glutathione. Ketones can protect neurons against dysfunction and degeneration in animal models of PD. (Mattson MP, Ageing Res Rev, 2015; Phillips MCL, Mov Disord, 2018; Shaafi S, Iran J Neurol, 2016; Cheng B, Brain Res, 2009)
- Exercise: Numerous meta-analyses and systematic reviews show that resistance training, dance (particularly tango) and Tai Chi improved gait velocity, balance and symptom scores, while aerobic exercise improved memory and executive function and increased cerebral gray matter volume (Tang L, J Clin Neurosci, 2019; Flynn A, J Physiother, 2019; Stuckenschneider T, J Parkinsons Dis, 2019; Ni M, Neurorehabil Neural Repair, 2018; da Silva FC, PLoS One, 2018; Ahlskog JE, Neurology, 2011).
- Hyperbaric oxygen: No human studies but can inhibit the decrease in rat dopaminergic neurons (Kusuda Y, Neurosci Res, 2018).
- Near infrared radiation: Red and infrared light helmets induced symptom improvement, as assessed by the patients. In animals, infrared increased dopaminergic cells and locomotor activity and reduced behavioural impairment and astrogliosis (Hamilton CL, Photobiomodul Photomed Laser Surg, 2019; Reinhart F, Neurosci Res, 2017; El Massri N, Exp Brain Res, 2016; Reinhart F, Exp Brain Res, 2016; Darlot F, 2016 Ann Neurol).

Mitochondrial remedies for which there is animal or in vitro evidence for PD (Annex C)

- Astaxanthin
- B vitamins
- Cannabinoids
- L-carnitine
- Coenzyme Q10
- Creatine
- Lipoic acid
- Melatonin
- Omega 3 fatty acids
- Vitamin D

Flavonoids and isoflavones

- Baicalin/Baicalein
- Curcumin
- Epigallocatechin-3-gallate (EGCG)
- Quercetin

Flavonoids

A combination works best, as this is how they arise in nature. Their individual effects complement each other.

Multiple sclerosis (MS) and mitochondria

- Multiple sclerosis is a demyelinating disease in which the insulating covers of neurons in the brain and spinal cord are damaged, disrupting the ability of parts of the nervous system to communicate, with gradual deterioration of neurological function and axonal dysfunction and degradation.
- As an autoimmune disease, there is an important immune component. Patients with progressive forms of MS exhibit a sustained increase in the number of Th1, T cytotoxic type-1 and Th17 cells in peripheral blood, suggesting a permanent peripheral immune activation. T cells from progressive patients showed lower oxygen consumption rate, mitochondrial mass, membrane potential and respiration and a higher tendency to shift towards glycolysis upon stimulation with upregulated glucose transporters and glycolytic-supporting genes.
- Several studies have demonstrated mitochondrial ETC deficiency in MS, with a reduction in the activities of Complexes I and III, as well as mtDNA deletions and mutations, and abnormalities in mitochondrial transport, creating an energy imbalance and contributing to progressive neurodegeneration and irreversible disability.
- There are mitochondrial changes in axons lacking healthy myelin sheaths, as well as redistribution of sodium channels, suggesting that demyelinated axons would be more vulnerable to energy deficits than myelinated axons. The axons with dysfunctional or absent Na+/K+ ATPase are no longer be able to efflux sodium, maintain resting membrane potential or conduct nerve impulses.

(Kamat PK, Cell Biochem Biophys, 2014; Nissanka N, FEBS Letts, 2018; De Biasi S, Eur J Immunol, 2019; Barcelos IP, Biology, 2019)

Treatment of multiple sclerosis with mitochondrial therapies

- Caloric restriction (CR) or fasting: A pilot study found that both 22% daily CR and 75% CR 2 days/week showed significant improvements in emotional well-being/depression scores (Fitzgerald KC, Mult Scler Relat Disord, 2018). CR also induced remyelination in mice (Mojaverrostami S, Metab Brain Dis. 2020).
- Ketogenic diet: An MCT-based ketogenic diet induced no clinical improvement, although other studies found a reduction in pro-inflammatory enzymes and normalisation of the colonic microbiome after 6 months (Lee JE, J Am Coll Nutr, 2020; Bock M, EBioMedicine, 2019; Swidsinski A, Front Microbiol, 2017; Choi IY, Cell Rep. 2016; 15(10):2136-2146).
- Exercise: Meta-analyses found that most exercise was beneficial for fatigue, particularly swimming, while both aerobic and resistance training were equally highly effective in improving lower extremity physical function and perceived fatigue (Chen Y, Int J Sports Med. 2021; Taul-Madsen L, Arch Phys Med Rehabil. 2021).
- Hyperbaric oxygen: A Cochrane Review found no benefit (Bennett M, Cochrane Database Syst Rev. 2004).
- Therapeutic hypothermia: No studies
- Infrared therapy: Benefited IL-10 levels in humans and reduced the clinical score in mice (Silva T, PLoS One. 2020; Gonçalves ED, Autoimmunity, 2016)
- Pulsed electromagnetic fields: Reduced pain in humans (Hochsprung A, Neurologia, 2021).

Treatment of MS with mitochondrial remedies (Annex C)

- B vitamins
- Cannabinoids
- Coenzyme Q10

- Lipoic acid
- Omega 3 fatty acids
- Vitamin A *

Vitamins and minerals with *
All essential for mitochondrial function but in excess can be highly toxic.
Both mitochondrial deficiency and excess induce mitochondrial damage.

Despite low vitamin D being a risk factor for MS, supplementation did not improve clinical outcome or relapse rate.

Amyotrophic lateral sclerosis (ALS) (aka motor neurone disease) and mitochondria

- ALS causes the death of motor neurons controlling voluntary muscles, located in the motor cortex, brainstem and spinal cord. It is characterised by stiff muscles, muscle twitching and gradually worsening weakness due to muscles decreasing in size. 5-10% of cases are familial, mostly associated with mutations in mtSOD which allows increased ROS; the remainder are idiopathic.
- Mitochondrial dysfunction is involved in the pathogenesis of ALS, probably by inducing calcium-mediated excitotoxicity in motor neurons through increasing generation of ROS and by initiating apoptosis. Axonal transport of mitochondria along cytoskeletal tracks is disrupted in ALS, particularly familial ALS, and may also impact mitochondrial fission and fusion. In addition, histology in ALS patient spinal cords showed a specific neuronal decrease in cytochrome oxidase activity.
- Impaired activity of Complex IV has also been established in the brains of patients with ALS; respiratory chain defects are observed prior to disease onset, suggesting a possible role in pathogenesis.

(Ansari A, Aging Cell, 2017; Duchen MR, Eur J Physiol, 2012; Kamat PK, Cell Biochem Biophys, 2014; Nissanka N, FEBS Letts, 2018; Burchell VS, Expert Opin Ther Targets, 2010)

‘Motor neurone disease: Edinburgh scientists reveal breakthrough’ BBC, January 2021

- ‘Scientists are a step closer to being able to reverse the damage caused by motor neurone disease (MND).’
- ‘The research found that the damage to nerve cells caused by MND could be repaired by improving the energy levels in mitochondria.’
- ‘They discovered in human stem cell models of MND, the axon - the long part of the motor neuron cell that connects to the muscle - was shorter than in healthy cells.’
- ‘And the movement of the mitochondria, which travel up and down the axons, was impaired.’
- ‘The scientists showed that this was caused by a defective energy supply from the mitochondria and that by boosting the mitochondria, the axon reverted back to normal.’

(Mehta AR, Acta Neuropathol, 2021)

Mitochondrial therapies in ALS

- Ketogenic diet, fasting, caloric restriction: Insufficient evidence.
- Exercise: A systematic review showed that therapeutic physical exercise could contribute to slowing the deterioration of the musculature of ALS patients, thus facilitating their performance in activities of daily living, in the short, medium and long term (Ortega-Hombrados L, Int J Environ Res Public Health. 2021).
- Hyperbaric oxygen: insufficient evidence
- Therapeutic hypothermia: insufficient evidence
- Near infrared radiation: insufficient evidence
- Pulsed electromagnetic fields: insufficient evidence

Mitochondrial remedies in ALS

Insufficient evidence for any.

Huntington's disease (HD)/Huntington’s chorea and mitochondria

- HD is a mainly autosomal dominant disorder caused by a mistaken trinucleotide repeat sequence in the huntingtin (htt) gene (known as a ‘genetic stutter’) resulting in excess glutamine being added to the htt protein. This leads to the progressive degeneration of neurons, which impacts functional abilities, usually resulting in movement, cognitive and psychiatric disorders and leading to cognitive decline and dementia. Oxidative damage has been implicated in Huntingdon’s disease, where overexpression of mutant htt sensitises cells to oxidative stress. Several biomarkers for oxidative stress were found in post mortem patients as well as htt fragments in brain mitochondria. Antioxidant status was also altered, with decreased brain glutathione but upregulation of endogenous antioxidants.
- Mutant huntingtin can interact directly with the outer mitochondrial membrane and inhibits PGC-1a expression, allowing reduced membrane potential and depolarisation at lower calcium loads compared to normal. Inhibition of mtPTP opening could delay this effect. The balance between neuronal fusion and fission is again aberrantly shifted toward huntingtin-associated fission, resulting in mitochondrial fragmentation. Huntingtin is also associated with impaired neuronal mitochondrial trafficking.
- Aconitase, a TCA cycle enzyme, is routinely found to be downregulated through oxidation, probably due to its Fe-S clusters. Iron-containing proteins are altered in HD, with succinate dehydrogenase, the main component of Complex II, displaying decreased expression of its Fe-S subunit in human post-mortem tissue. HD is linked to reduced Complex II and Complex III activity in the striatum. Chemical inhibition of Complex II induces HD-like symptoms in animals.
- Decreased glucose but increased lactate levels in the brain suggest impaired energy metabolism. Since this is found in pre-symptomatic carriers, it suggests that mitochondrial dysfunction is an early defect in the disease. Respiratory chain defects are also observed prior to disease onset, suggesting a possible role in pathogenesis. Similarly, early-stage HD patients present decreased PGC-1a mRNA levels in the striatum, with correlation between PGC-1a reductions and mitochondrial reductions in HD post-mortem brains.

(Duchen MR, Eur J Physiol, 2012; Franco-Iborra S, Front Neurosci, 2018; Kamat PK, Cell Biochem Biophys, 2014; Burchell VS, Expert Opin Ther Targets, 2010; Franco-Iborra S, Front Neurosci, 2018; Batlevi Y, Neurobiol Dis, 2011; Ansari A, Aging Cell, 2017)

Mitochondrial therapies in Huntingdon's

- Ketogenic diet, fasting, caloric restriction: insufficient evidence.
- Exercise: insufficient evidence
- Hyperbaric oxygen: insufficient evidence
- Therapeutic hypothermia: insufficient evidence
- Near infrared radiation: insufficient evidence
- Pulsed electromagnetic fields: insufficient evidence

Mitochondrial remedies in Huntingdon's

- Insufficient evidence for any.