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New Therapeutics to Modulate Mitochondrial Function in Neurodegenerative Disorders

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Abstract

Background—Mitochondrial function and energy metabolism are impaired in neurodegenerative diseases. There is evidence for these functional declines both within the brain and systemically in Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis. Due to these observations, therapeutics targeted to alter mitochondrial function and energy pathways are increasingly studied in pre-clinical and clinical settings.

Methods—The goal of this article was to review therapies with specific implications on mitochondrial energy metabolism published through May 2016 that have been tested for treatment of neurodegenerative diseases.

Results—We discuss implications for mitochondrial dysfunction in neurodegenerative diseases and how this drives new therapeutic initiatives. Conclusion: Thus far, treatments have achieved varying degrees of success. Further investigation into the mechanisms driving mitochondrial dysfunction and bioenergetic failure in neurodegenerative diseases is warranted.

Keywords

Mitochondria; Alzheimer's disease; Parkinson's disease; Amyotrophic Lateral Sclerosis; Bioenergetics; Oxidative Stress; neurodegeneration

INTRODUCTION

Despite decades of research, the underlying etiology of the most common neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and Amyotrophic Lateral Sclerosis (ALS) remains unknown. This is possibly because the cause of these disorders involves a combination of both environmental and genetic factors. By definition, neurodegenerative diseases involve the loss of neurons, cells unique in their role of transmitting information through chemical and electrical signals. This transmission of information requires high energy demand, and it is often cited that while comprising only 2% of an individual's body weight, the brain consumes 20–25% of the body's oxygen.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

Maintenance of ion gradients for neurotransmission is the primary source of this energy demand, with half of the brain's energy allotment used to maintain membrane potentials [1]. Thus, a great deal of current research into therapeutics targeting neurodegeneration involves brain metabolic processes.

Glucose is an essential source of energy for neuronal tissues. *In vivo* declines in glucose metabolism, as measured by Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET), are observed in AD, PD (including non-demented PD subjects), and ALS, providing additional evidence for bioenergetic failure in clinical neurodegenerative diseases [2–4]. Glucose catabolism provides key intermediates for the citric acid cycle (or Krebs's cycle, tricarboxylic acid cycle; TCA), where the conversion of pyruvate (or other substrates) to NADH and FADH₂ fuels substrate level oxidative phosphorylation and the generation of ATP (Fig. 1); see Magistetti *et al.* for an excellent review on brain energy metabolism [5]. These bioenergetic pathways within the mitochondria are the most efficient way to generate energy, while at the same time giving rise to a variety of physiologically-relevant intermediate molecules. Thus, it is not surprising that mitochondrial dysfunction is widely-recognized as a common theme to neurodegenerative diseases. The current review will address mitochondrial dysfunction as a common contributing factor to neurodegenerative diseases. Further, novel therapeutics that target mitochondrial function and their mechanisms of action will be addressed.

ALZHEIMER'S DISEASE

AD is the most common neurodegenerative disease. Clinical diagnosis is confirmed at autopsy by the presence of cerebral amyloid beta (A β) plaques and tau tangles. Both postmortem pathology and genetic studies have fueled the development of the A β cascade hypothesis. This hypothesis asserts that A β drives neurodegeneration in AD. While this hypothesis is still widely accepted, and A β still has diagnostic utility, a primary role of A β in AD etiology is beginning to be questioned. This is in part due to several Phase 3 clinical trials targeting A β that failed to affect primary outcomes of clinical or neuroimaging measures of disease progression [6–10].

In light of these recent findings, the role of mitochondrial dysfunction in AD etiology is increasingly being considered. The mitochondrial cascade hypothesis proposes that functional deficits occur early in mitochondria, preceding and driving the observed A β and tau pathologies [11–13]. In preclinical models, it has been shown that reactive oxygen species (ROS) from mitochondria can enhance A β production [14]. Mitochondrial deficits occur prior to A β accumulation in AD transgenic mouse models [15]. In human studies, AD subjects are known to exhibit systemic mitochondrial deficits [16, 17]. Here, we discuss the relevance of mitochondrial dysfunction to the pathological hallmarks of AD.

Cytoplasmic Hybrid (Cybrid) Studies

Studies of cytoplasmic hybrid (cybrid) cells suggest mitochondrial DNA (mtDNA) drives pathological processes observed in AD. Cybrid cells provide a model to study the contribution of mtDNA to specific disease hallmarks. Cybrid cells are generated by transferring patient-specific cytoplasts (usually platelets) to a cell lacking mtDNA (p0) (Fig.

2). Over time, the only known perpetuating material within the cytoplasm (mtDNA from the subject) remains, with a constant nuclear background [18].

Several studies have examined the contributions of mtDNA from sporadic AD subjects to AD-relevant pathologies. Cybrids generated from AD subjects have reduced cytochrome oxidase (Complex IV, COX) activity, increased ROS, and decreased mitochondrial membrane potential [19]. AD cybrids recapitulate changes in A β as well, with higher levels of secreted A β ₁₋₄₀ and A β ₁₋₄₂, elevated intracellular A β ₁₋₄₀, and Congo red positive A β deposits. Increased markers of apoptosis are also observed, including the presence of cytoplasmic cytochrome c and caspase-3 activation [19]. This suggests mtDNA may play an important role in driving pathological changes observed in AD.

Independent studies of AD cybrids have recapitulated many of these observations, including reduced COX activity [17, 20]. The observed changes in mitochondrial function are worsened with higher passage number in AD cybrids [20]. Further, changes can be observed not only in cybrids generated from AD subjects, but also those from subjects diagnosed with mild-cognitive impairment (MCI). Cybrids generated from AD and MCI subject platelet mtDNA had reductions in mitochondrial respiration with an increase in the mitochondrial leak rate, as well as reduced glycolysis. This manifested in lower NAD/NADH, but higher ADP/ATP ratios. However, mitochondrial mass appeared increased, and proteins which control mitochondrial fission were elevated in the AD cybrids [17]. A detailed review of cybrid studies is available elsewhere [18].

Pre-Clinical AD Models

Mitochondrial dysfunction is evident in pre-clinical AD mouse models, and appears to precede other pathological hallmarks. In the 3XTg-AD (transgenic mice bearing Swedish mutant amyloid precursor protein (APP)- K670N/M671L, tau mutant-P301L, and presenilin mutant-M146V) mouse model, mitochondrial respiration was reduced and ROS was increased early, prior to changes in A β [15]. This is further exemplified by observations of mitochondrial dysfunction in hippocampal neurons derived from embryonic mice. These data are consistent with mitochondrial dysfunction preceding brain A β deposition [15].

Further studies have examined the relationship between A β levels and mitochondrial dysfunction. Mice bearing the Swedish APP mutation (K670N/M671L) alone or in combination with mutant presenilin (M146V) mitochondria in the hippocampus and cortex show increased markers of damage and dysfunction. High A β is observed in these regions, with the most severe deficits in synaptic mitochondria [21]. In a separate study, synaptosomal mitochondria were significantly impaired in the 5xFAD (transgenic mice bearing Swedish, Florida, and London mutations in APP- K670N/M671L, I716V, V717I; with two presenilin mutations-M146L and L286V) mouse model. Mitochondria at synaptosomes were skewed towards increased fission, with elevated markers of autophagy. These observations correlated with memory impairment. Synaptosomal mitochondria had a reduced respiratory control ratio and state III respiration, with reduced ATP production [22]. These findings are important, because mitochondria localized to synaptosomes are critical to neuronal function and signal transduction.

A strong relationship between mitochondrial dysfunction and APP exists. Full-length APP protein is trafficked to mitochondria, where it associates with mitochondrial translocase machinery. APP may prohibit protein import into mitochondria, leading to mitochondrial dysfunction. In models where APP is localized to mitochondria, reductions in COX activity, reduced ATP production, and depolarized mitochondrial membrane potential were observed. Localization of APP to mitochondria occurs *in vitro*, *in vivo*, and in AD human subjects [23, 24]. Enzymes which process APP into its fragments are also found within mitochondria. A functional γ -secretase was shown to cleave mitochondria-localized APP into the P3, APP intracellular domain (AICD), and possibly A β fragments [25]. Further, in the 5xFAD mouse model, the C99 APP fragment localized to mitochondria and this could be modulated by a heterozygous deletion of β -secretase (BACE) [26]. Overall, data suggest APP localizes to mitochondria where it can be processed into C-terminal fragments, resulting in P3, AICD, and A β generation within mitochondria. These findings are further supported by studies where A β is localized to mitochondria and is associated with mitochondrial dysfunction [21, 27, 28]. However, a classical question remains, is A β causing mitochondrial dysfunction or is mitochondrial dysfunction causing A β ?

AD Human Studies

Mitochondrial dysfunction is observed in human AD subjects, both within the brain and systemically. Pronounced metabolic deficits are first observed in the posterior cingulate, prior to dementia onset. Early FDG-PET imaging shows reduced glucose metabolism in the posterior cingulate cortex and cingulate parietal transitional area very early in clinical AD [29]. These metabolic changes are accompanied by reductions in mitochondrial COX activity. In a post-mortem study, quantitative immunochemistry staining for COX showed a significant reduction in AD subjects within six layers of the posterior cingulate [30]. An additional study observed reductions in COX activity in the frontal, temporal, and parietal cortex [31].

Numerous studies have replicated the finding of decreased COX activity in AD subject post-mortem brain. One study found COX activity is significantly decreased in the frontal, temporal, parietal, and occipital cortex. Complex I and Complex II–III activities were slightly decreased but only in the occipital cortex [32]. A separate study found a non-significant decrease in Complex I and II in AD brain postmortem. A decrease in Complex III was found, but the most striking ETC deficit was in COX [33], while cytochrome aa₃, b, and c levels were unchanged. Immunohistochemical analyses revealed that AD subjects had higher levels of COX-deficient neuronal cells in the hippocampus. A similar effect was observed in epithelial choroid cells [34]. Further analysis of single COX-deficient neurons found increased mtDNA deletions [35]. Studies have also shown abnormal kinetic properties of the COX enzyme in AD brain postmortem. At low substrate levels, a K_m (substrate concentration at half the maximum velocity-Michaelis constant) was not identifiable. However, at high substrate levels the K_m in the AD COX samples was higher than the control [36]. The underlying cause for these observations is unknown.

Beyond brain tissue, COX activity is reduced in AD subject platelets. [16, 37]. While COX activity is lower, COX expression is unchanged, ATP levels are reduced, and ROS are higher

in platelets from AD subjects [37]. Since COX directly provides electrons to ATP synthase (or Complex V), reductions in COX activity could lead to impaired ATP production (Fig. 1). One study found reduced COX activity in AD subject platelets, but no significant effects were observed for other ETC complexes including Complex I and Complex II [16].

A mitochondrial etiology of AD is further exemplified by an association of AD risk with maternal inheritance. mtDNA is almost entirely derived maternally, and those whose mother is afflicted with AD have a higher risk to develop AD [38, 39]. Early AD-associated changes are observed in individuals whose mother, but not father, have AD. These include aberrant brain glucose metabolism, decreased platelet COX activity, and decreased gray matter volume [40–43]. Given the emerging role of mitochondria and energy metabolism in AD pathogenesis, development of new therapeutic strategies which target mitochondria is imperative. A summary of some of the primary mitochondrial mechanisms that are currently being targeted is given in Fig. (3). These targets are described in more detail below.

MITOCHONDRIAL MEDICINE AND ALZHEIMER'S DISEASE

Diet

Mitochondrial medicine can include both pharmacological and lifestyle interventions. One such lifestyle intervention is diet. Brain energy metabolism, including mitochondrial function, is altered with the ketogenic diet in pre-clinical mouse models [44–46]. Caloric restriction alters energy pathways through activation of sirtuin 1 (SIRT1), which can initiate downstream mitochondrial biogenesis [47]. Other dietary factors and diets, such as fatty acids and the Mediterranean diet may modulate AD risk. Evidence for diet-based therapeutics in modulating AD pathology are discussed below.

Mediterranean Diet—Both the Mediterranean-Dietary Approach to Stop Hypertension (MIND) and the Mediterranean diet are associated with a decreased risk of developing AD [48, 49]. The Mediterranean diet is rich in fruits, vegetables, nuts, fish, and olive oil. The MIND diet is very similar and includes foods such as olive oil, green leafy vegetables, whole grains, nuts, berries, beans, fish, and poultry. Individuals participating in this diet are also encouraged to avoid sweets (such as pastries), cheese, red meat, butter, and margarine. In addition to decreased risk for AD, subjects who adhere to the Mediterranean diet had increased cortical thickness in brain regions afflicted by AD, including the entorhinal, orbitofrontal, and the posterior cingulate cortex [50].

Fatty Acids

Specific dietary components, such as fatty acids, have also been assessed in relation to AD. While omega-3- fatty acids did not alter the rate of cognitive decline (as measured by mini-mental state exam or MMSE) in moderate to severe AD subjects, a significant slowing of cognitive decline was found in subjects who had mild AD [51]. The omega-3- polyunsaturated fatty acid, docosahexaenoic acid (DHA) was beneficial to the Tg2576 (transgenic mouse overexpressing Swedish mutant APP- KM670/671NL) AD mouse model. Tg2576 mice that received a diet highly enriched with DHA had less A β _{1–40} and A β _{1–42}, reduced plaque burden, reduced APP C-terminal fragments, and altered APP cellular

localization [52]. A separate pre-clinical study combined DHA with either peptamen (high protein supplement) or a low-fat diet in the TgCRND8 (transgenic mouse overexpressing Swedish and Indiana mutant APP- KM670/671NL and V717F) AD mouse model. Peptamen in combination with DHA increased A β levels and plaques, while the low-fat diet in combination with DHA reduced A β levels and plaques [53]. These data suggest it is important to study how different dietary additives interact.

DHA is an important dietary consideration throughout lifespan, with regards to cognitive function and overall brain health [54]. In middle aged, cognitively-healthy subjects, DHA was related to better performance on tasks such as working memory, vocabulary, and nonverbal reasoning [55]. Higher consumption of DHA has also been linked to reduced AD risk, although there is disparity regarding DHA blood levels and risk of dementia [56–58]. Further, in a randomized, placebo-controlled clinical trial, DHA had no effect on cognitive decline in AD subjects [59].

An additional fatty acid, Oleic acid, is linked to longer life span in *C. elegans* [60]. Oleic acid may also alter APP processing, and has been shown to reduce secreted A β in Cos-7 cells overexpressing APP. Transgenic mice expressing a mutant human APP (Swedish and Indiana mutations- KM670/671NL and V717F) fed a low-fat cholesterol free diet enriched with Oleic acid, displayed decreased A β plaques, reduced BACE and presenilin, and elevated levels of soluble APP α (sAPP α) [61]. These data suggest that Oleic acid alters APP processing both *in vitro* and *in vivo*, however to date no clinical studies have tested the efficacy of Oleic acid on cognition in either cognitively normal or AD subjects.

Caloric Restriction—Caloric restriction is known to increase life span and insulin sensitivity across several species and may be modulated in part by ROS and/or the activity of an enzyme family called the sirtuins [62–66]. Caloric restriction also alters APP processing. In particular, caloric restriction lowered A β production, decreased A β plaques, increased sAPP α levels, and increased the activity of α -secretase, in the Tg2576 AD mouse model [67]. Positive benefits were also observed in squirrel monkeys with caloric restriction; A β _{1–40} and A β _{1–42} were reduced in the temporal cortex, with no change in APP expression. This study also observed caloric restriction increased α -secretase activity [68]. While clinical studies examining caloric restriction in subjects with MCI are ongoing, completed studies have not yet published results (clinicaltrials.gov IDs NCT01286389, NCT01219244, NCT01504854).

Ketogenic Diet—Other dietary measures have proven beneficial in neurological disorders, such as the ketogenic diet in epilepsy [69, 70]. The ketogenic diet has further shown promise in AD pre-clinical models. For example, female mice bearing the Indiana (V717I) APP mutation had reduced brain A β levels, but no change in behavioral outcomes when fed a ketogenic diet [71]. In the APP/PS1 knock-in AD mouse model, the ketogenic diet had no effect on A β levels in the brain or skeletal muscle, but these mice did show significant improvement on the rotarod motor performance test [72]. Using the APP^{swe}/PS1^{dE9} mouse model (transgenic mice expressing Swedish APP mutations- KM670/671NL and PSEN1 delta E9), a ketogenic diet in combination with an anaplerotic compound, triheptanon (a triglyceride composed of three, seven carbon fatty acids), improved memory test

performance, and reduced markers of neuroinflammation. However, this combination therapy did not alter A β levels or plaques within the brain [73]. In the 3XTgAD mouse model, a diet enriched for ketone-esters led to subtle memory and learning performance improvement, decreased A β deposition, and reduced brain burden of phosphorylated tau [74].

Dietary considerations for the treatment of AD may need to take into account apolipoprotein E (ApoE) genotype. Pre-clinical studies of mice bearing ApoE ϵ 3 or ApoE ϵ 4 alleles were found to show disparate effects with various diets. Such that, the high fat diet and ketogenic diet increased plasma levels of ApoE in the ApoE ϵ 4 mice. However, the high fat diet decreased hippocampal levels of ApoE in the ApoE ϵ 3 mice, and the ketogenic diet showed no effect on ApoE levels in these mice [75]. Overall, this may be an important consideration in future clinical study design.

The efficacy of the ketogenic diet in Alzheimer's disease currently being examined. Further, the effects of the ketogenic diet in cognitively normal, elderly subjects is also underway in clinical trials. As most of these current studies are still recruiting and/or ongoing, results are not available (clinicaltrials.gov IDs NCT02521818, NCT02709356, NCT02551419, NCT02409784).

Exercise

Exercise is known to increase peripheral aerobic capacity, likely through effects on mitochondria by several mechanisms. Exercise induces acute mitochondrial biogenesis, which is maintained through increased expression of upstream PGC1- α regulators [76–78]. Moreover, both expression of ETC complexes and mitochondrial coupling efficiency are increased in active compared to sedentary subjects [79]. Aerobic capacity declines with age and mitochondrial mechanisms, such as uncoupling, are postulated to underlie declines in exercise efficiency in the elderly [80, 81]. These mechanisms could be further exacerbated by disease, as AD subjects exhibit both peripheral and central markers of mitochondrial dysfunction [17, 82, 83].

Pre-Clinical Studies—Pre-clinical studies support potential benefits of exercise in AD. Voluntary exercise improved learning and memory and decreased A β plaques within the frontal cortex and hippocampus of TgCRND8 AD mice. Exercise was postulated to influence APP processing, because APP C-terminal fragments were reduced [84]. More recent studies in Tg2576 AD mice also found that exercise mitigated brain levels of A β _{1–42}, and in APPswe/PS1dE9 mice, treadmill exercise was associated with improved spatial memory, more Brain-Derived Neurotrophic Factor (BDNF) positive cells and less activated microglia in the brain, but no change in brain A β [85, 86].

There is also evidence that exercise may modulate tau phosphorylation. In the Tg601 mouse model (which overexpresses wild-type human tau), treadmill exercise training increased tau phosphorylation and exacerbate neuroinflammation [87]. However, this effect may be dependent upon the mouse model studied, as exercise has also been shown to mitigate inflammatory markers in mouse blood and cortex [88].

The mechanism of exercise benefits may involve several factors that converge to increase hippocampal neurogenesis, including neurotrophins, lactate, and mitochondrial function. In a rat model of vascular dementia, exercise increased neural progenitor cells in the subgranular zone of the hippocampus and elevated the expression of BDNF [89]. Further studies have shown that exercise above the lactate threshold, in wild-type aged mice (C57BL/6) resulted in increased hippocampal Vascular Endothelial Growth Factor-A (VEGF-A) expression, and brain expression of mitochondrial biogenesis genes. A negative correlation between doublecortin (a neurogenesis marker) and C-C motif chemokine 11 (CCL11) expression was also observed [90]. Lactate may mediate some of the changes observed with exercise. Both lactate and exercise facilitated increased expression of genes involved in mitochondrial biogenesis (PGC1 related co-activator protein, PRC) and increased VEGF-A gene expression within the brain [91]. The overall mechanism exercise mediated brain benefits warrants more investigation.

Clinical Studies—Exercise has shown positive effects in clinical studies of older cognitively normal adults. In a questionnaire based study, exercise was associated with decreased cerebral amyloid and higher $A\beta_{1-42}$ CSF concentrations. Exercise appeared to associate more with these biomarkers in ApoE $\epsilon 4$ non-carriers [92]. However, no association between exercise and CSF tau or phosphorylated-tau 181 was observed. Dual task training (simultaneous cognitive-motor task and exercise), improves cognition (mini mental state examination) and decreases the plasma $A\beta_{42/40}$ ratio [93]. The most commonly noted effect of physical activity is on the domain of executive function, an effect postulated to be mediated through change in aerobic capacity (VO_2 Max) [94]. Finally, increased change in aerobic capacity due to exercise (1 year of walking) has also been related to significant increases in hippocampal volume [95]. These studies indicate exercise training is beneficial to elderly cognitively normal individuals.

Clinical studies have shown positive effects of exercise in cognitively-impaired subjects. A large, randomized controlled trial found that cognitively-impaired subjects who exercised aerobically had greater memory improvement than an education-only control group [96]. Another study of a multi-component exercise program showed improved memory and decreased brain atrophy in subjects with amnesic, but not non-amnesic MCI compared to an education only control group [97]. Other studies indicate positive effects on other areas, such as executive function, cognitive, or quality of life outcomes although not all studies are consistent [98–102]. The relationship between exercise and cognition appears to be dependent upon sample size and the sensitivity of the cognitive and neuroimaging outcome measures, although the effect of the intervention method (i.e. aerobic vs. resistance training) and the control group (education only vs. active control) warrant further exploration.

Meta analyses generally have shown a benefit of physical activity and cognition. In a meta-analysis of 18 randomized control trials for physical activity and cognitive function, a positive effect was found on cognitive function. This included aerobic only or aerobic in combination with non-aerobic exercise, at low or high frequencies, and for subjects with or without AD [103]. A separate systemic review of 13 studies, reported overall beneficial effects of exercise on cognition, neuropsychiatric scores, daily tasks, and functional capacity in AD subjects [104]. Finally, a meta-analysis of only randomized controlled trials in MCI

and AD revealed exercise resulted in moderate to strong effects on cognitive function in AD subjects, but smaller effects in MCI subjects [105]. Similar to pre-clinical studies, exercise increases plasma levels of BDNF in healthy and AD subjects [106]. It has been hypothesized that exercise provides beneficial effects through increasing the action of BDNF, improving mitochondrial function and glucose metabolism, thus decreasing ROS and increasing the rate of removal of damaged mitochondria. This overall could decrease the production of mitochondria-derived damage associated molecular pattern (DAMP) molecules and sterile inflammation [107]. Additional pre-clinical and clinical studies will further the understanding surrounding the mechanism of exercise benefits to the brain.

Diet and Exercise

Combining exercise and diet interventions may further impact beneficial outcomes in AD subjects. Combined omega-3-fatty acid supplementation with aerobic exercise in subjects with MCI eliminated the gray matter volume decreases within the frontal, cingulate, and parietal cortex observed in individuals who did not receive the combined treatment. However, no change in cognitive performance, inflammatory, vascular, or metabolic markers were observed between groups [108]. Expanded efforts into combination therapeutic strategies are warranted.

Metabolic Hormones

In AD transgenic mice, diet-induced insulin resistance increased A β plaque burden [109]. This is consistent with clinical studies showing that insulin resistance increases AD risk [110]. Insulin and a related network of peripherally secreted “incretin” hormones including glucagon-like peptide-1 (GLP-1), and Gastric inhibitory polypeptide (GIP; also known as glucose-dependent insulinotropic peptide) can cross the blood brain barrier and have receptors in the hippocampus [111–115]. Insulin resistance has been associated with mitochondrial dysfunction, although the casual relationship between these two processes is unclear [116]. Intranasal insulin has shown some promise in improving memory in humans although insulin sensitizers have not and this has been reviewed elsewhere [117–123]. Incretins are also associated with mitochondrial function and both GLP-1 and GIP have shown neuroprotective properties in preclinical AD models [124–126]. In particular, the molecule Liraglutide (an agonist of the GLP-1 receptor) is protective of mitochondria in cell culture and mouse models [127, 128]. This compound is currently being investigated in clinical trials of AD subjects. One trial that examined the effect of Liraglutide on cerebral amyloid has been completed but results are not yet available ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01469351) ID NCT01469351) and another that aims to test the effect on cerebral metabolism and cognitive change is ongoing ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01843075) ID NCT01843075).

Oxaloacetate

Oxaloacetate (OAA), a molecule central to several bioenergetic pathways, is a novel therapeutic approach for AD. OAA can be reduced to malate enzymatically within the cytoplasm. This reaction allows malate to be transported into mitochondria to provide substrates for the TCA cycle. Further, an enzyme within the mitochondria can oxidize malate to generate OAA. OAA can also be converted to phosphoenolpyruvate (PEP) within the cytoplasm as a part of gluconeogenesis. *In vitro* studies have found that OAA supports

glycolysis and mitochondrial respiration fluxes, promotes expression of mitochondrial biogenesis proteins (PGC1 α) and enhances SIRT1 phosphorylation [129]. *In vivo* studies have shown that intraperitoneal injection of OAA in 4-month old C57Bl/6 male mice for two weeks enhances brain bioenergetic pathway protein expression and neurogenesis markers. OAA also increased the expression and nuclear localization of PGC1 α and increased AKT signaling. OAA also increased the expression of doublecortin (a neurogenesis marker) and increased the number of doublecortin positive cells within the hippocampus of C57Bl/6 mice [130]. Currently, a clinical trial examining OAA in AD subjects is ongoing (clinicaltrials.gov IDs NCT02063308, NCT02593318).

Antioxidants

Free radicals are endogenously produced as a by-product of bioenergetic pathways [131, 132]. If allowed to go unchecked oxidative and nitrosative free radicals (such as superoxide, hydrogen peroxide, and peroxynitrite) damage cellular macromolecules. Within mitochondria, free radicals can compromise lipid membranes, ETC enzyme complexes, and DNA [133]. This can lead to the initiation of cell death cascades, such as apoptosis or necrosis [134, 135]. Whether this mechanism is a cause or effect in AD, remains to be determined. However, mitigating oxidative stress with antioxidants, may prove beneficial in AD outcomes.

Vitamin E—Vitamin E (or α -tocopherol) is a well-studied insoluble, lipophilic antioxidant. The effects of vitamin E have been addressed across numerous neurodegenerative diseases. Vitamin E dietary intake alters cognitive performance in AD pre-clinical models. Rendering mice deficient in vitamin E and folate in conjunction with an iron-enriched diet reduces cognitive performance. This effect was more profound in *ApoE* knockout mice [136]. Further, mice lacking the α -tocopherol transfer protein gene (*Ttpa* $^{-/-}$) become depleted of α -tocopherol (vitamin E). Crossing the *Ttpa* $^{-/-}$ mice with the Tg2576 AD mouse model, resulted in worsening of cognitive impairment and enhanced A β deposition in the brain. Supplementation with α -tocopherol (vitamin E) rescued these effects [137]. In the Tg2576 AD mouse model, Vitamin E supplementation beginning at five months of age reduced A β brain deposition and brain oxidative stress markers. However, beginning vitamin E supplementation at an older age (13 months) did not alter disease outcome [138]. A separate study found that vitamin E reduced amyloidosis after repetitive concussive brain injury in the Tg2576 AD mouse model [139].

Vitamin E dietary intake and plasma concentrations also correlate with AD risk in human subjects. For example, dietary intake of vitamin E, C, or β -carotene are associated with decreased risk of AD. Of the three supplements, vitamin E dietary intake appeared to be more protective against AD risk [140]. Reduced plasma levels of tocopherols (including vitamin E) increases the risk for MCI and AD, while high levels of plasma vitamin E reduce the risk of AD [141, 142]. However, the use of vitamin E as a therapeutic in human AD subjects is controversial. Some studies suggest vitamin E may be harmful, while others suggest it could be beneficial [143]. A study that assessed the effects of vitamin E alone and in combination with memantine revealed that vitamin E (but not a combination of vitamin E and memantine) reduced the decline functional ability (ADCS-ADL) over a period of about

2 years [144]. As reviewed in a meta-analysis, three studies evaluated the benefits of vitamin E in AD or MCI subjects. One study reported benefits ranging from fewer participants reaching death or institutionalization, as well as benefits in the CDR score. However, the other two studies failed to find positive benefits in measured outcomes, which included the MMSE score and conversion rate of MCI to AD [145]. Stratifying subjects as vitamin E responders and non-responders may provide evidence as to why vitamin E is beneficial in some studies, but not in others. Subjects who benefited cognitively from vitamin E also had reduced markers of oxidative stress (blood GSSG). Subjects who did not cognitively benefit from vitamin E did not have a reduction in oxidative stress. This suggests that in some subjects, vitamin E lowers oxidative stress biomarkers and slows the rate of cognitive decline, while in other subjects vitamin E fails to alter either [146]. Further studies to elucidate what factors determine which subjects will respond to vitamin E are necessary.

Coenzyme Q10 and MitoQ—Coenzyme Q10 (CoQ10, ubiquinone, or ubiquinol) is a fat soluble antioxidant with a structure similar to vitamins. CoQ10 is endogenously found within mitochondria where it is a component of the ETC. It accepts electrons from Complex I and/or Complex II, passing them to Complex III (Fig. 1). While Coenzyme Q10 showed promise in pre-clinical AD models, it has failed to show clinical efficacy for neurodegenerative disease. Based on this, a novel mitochondrial targeted antioxidant, called MitoQ was developed. MitoQ (mitoquinone mesylate: [10-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadienyl) decyl triphenylphosphonium methanesulfonate]) contains ubiquinone (CoQ10) conjugated to a triphenylalkylphosphonium (a lipophilic) cation.

MitoQ protected cells from A β toxicity *in vitro*, preserving neurite outgrowth and mitochondrial function [147]. Further studies have shown that MitoQ is protective in both *in vitro* and *in vivo* models of AD. In primary cortical neurons MitoQ protected from A β induced neurotoxicity, loss of mitochondrial membrane potential, and ROS production [148]. Further, female 3xTgAD mice treated with MitoQ displayed better cognitive performance, lessened oxidative stress, reduced levels of A β deposition, astrogliosis, and markers of cell death [148]. Thus far, MitoQ has not been tested in human AD subjects.

Szeto-Schiller (SS) Peptide Antioxidants—A well-recognized caveat to antioxidant treatment, is ensuring the antioxidants reach the appropriate cellular compartments. In particular, targeting antioxidants to the mitochondrial matrix, a major source of ROS production within the cell, may prove more efficacious with antioxidant therapies. While MitoQ is one such approach, Szeto-Schiller (SS) peptide antioxidants are a novel approach to ensure delivery of antioxidants to the mitochondrial matrix. SS peptides are small, cell-permeable peptides consisting of aromatic-cationic peptides, which are capable of scavenging hydrogen peroxide and peroxynitrite. The scavenging ability of SS peptides is attributed to a dimethyltyrosine residue. SS peptides, including SS-31 and SS-02 are protective in models of oxidative stress both *in vivo* and *in vitro*, these studies and mechanisms have been reviewed elsewhere [149, 150]. Furthermore, SS peptide antioxidants have proven beneficial to *in vitro* models of AD [151]. SS31 improved axonal mitochondrial transport and synaptic viability in the Tg2576 AD mouse model [147, 152]. To date, no human clinical trials are listed for SS peptide antioxidants.

Curcumin—Curcumin, a spice commonly found in curry, is a polyphenolic antioxidant. In the Tg2576 AD mouse model curcumin, at least at a low dose produced beneficial effects. These include reduced markers of brain oxidative stress and neuroinflammation, as well as reduced brain A β levels and plaque burden [153]. In a small pilot study of AD subjects, curcumin increased plasma vitamin E levels, but had no effect on cognitive performance [154]. An additional study, did not find positive outcomes in AD subjects with curcumin. This study also reported subjects had low levels of plasma curcumin, suggesting the bioavailability of curcumin is low [155]. Overall, more consideration into the design of how curcumin is studied may overcome these limitations. In particular, groups have targeted curcumin to the mitochondrial matrix using a similar approach used for MitoQ; conjugation of curcumin to the lipophilic triphenylphosphonium cation [156]. Further work to target curcumin to the mitochondrial matrix, has focused on using biodegradable, polymeric nanoparticles in addition to conjugation with the lipophilic triphenylphosphonium cation. This approach was shown to protect *in vitro* models from A β toxicity [157].

N-acetyl-cysteine—N-acetyl-cysteine (NAC) is a compound which provides precursors for glutathione (GSH) synthesis. GSH is an endogenous antioxidant, which is critical for mitochondrial function [158]. It can cross the blood brain barrier and has been shown to increase brain GSH levels [159]. *In vitro*, NAC lowers the transcription of the APP gene [160]. Further studies have shown NAC protects from A β induced toxicity, and reduces the amount of secreted A β *in vitro* [161]. NAC also lowered the amount of phosphorylated tau [161]. In the TgCRDN8 mouse model, NAC decreased A β levels [162]. *In vivo*, NAC protected mice which received intracerebroventricular injection of aggregated A β through a preservation of cognitive function, potentiation of oxidative stress markers (GSH and MDA), and preservation of ChAT activity [163]. While NAC has not been evaluated independently, it has been in a combination therapy clinical trial study. A randomized, double-blind study evaluated a combinatorial therapy of a “nutraceutical formulation” composed of NAC, folate, vitamin E, vitamin B12, s-adenosyl methionine, and acetyl-L-carnitine in AD subjects. Subjects which received this “nutraceutical formulation” showed improvements on the dementia rating scale [164].

α -Lipoic Acid— α -lipoic acid is a co-factor for several TCA enzymes, and while it is important for aerobic metabolism, it also acts as an antioxidant. α -lipoic acid treated Tg2576 AD mice, had improvements in learning but no effect on brain A β levels or plaque deposition [165]. Mixed outcomes have been reported in clinical studies of AD subjects with α -lipoic acid. In one study, subjects were divided into two groups, one group had AD and diabetes mellitus, the other group only had AD. In both groups, a subset of subjects showed cognitive improvement with α -lipoic acid, but not all subjects showed improvement [166]. An additional study evaluated α -lipoic acid in combination with omega-3-fatty acids. The primary outcome, an oxidative stress marker in urine, F2-isoprostane, showed no change in either group. The secondary outcome measures included MMSE, ADL/IADL, and ADAS-cog. The combination of α -lipoic acid and omega-3-fatty acids significantly improved MMSE and IADL outcomes over 12 months [167].

Stabilized NADH—NADH, is a central intermediate in metabolic pathways, including glycolysis, the TCA cycle, and oxidative phosphorylation. NADH is a redox pair with NAD⁺, where NADH is a reducing agent and NAD⁺ is an oxidizing agent. Stabilized oral NADH has been tested in two clinical trials for AD subjects. The first clinical study was an open-label design and reported that cognitive performance was improved based on the MMSE and GDS [168]. In a randomized, double-blind, placebo-controlled clinical trial in AD subjects, NADH supplementation appeared to slow the progression of cognitive decline (MMSE and MDRS), but no effect was observed with CDR, attention, or other memory performance tests [169].

Parkinson's Disease

PD is a chronic and progressive age-related neurodegenerative disease which primarily affects motor function. As the second most common neurodegenerative disease, PD is caused by the degeneration of dopaminergic neurons that project from the substantia nigra to the striatum. The primary PD symptoms include bradykinesia, muscle rigidity, gait disturbances, and resting tremor, although over the lengthy disease course, dementia (PD dementia) occurs in a substantial percentage of individuals [170]. The preclinical phase of PD is thought to last several decades, as the onset of motor symptoms only begins when striatal dopamine depletion is no longer compensated [171]. Clinical diagnosis occurs after motor symptom onset. The etiology of PD is unknown. However, age is the predominant risk factor and mechanisms involving mitochondrial dysfunction, oxidative stress, and neuroinflammation all contribute to neuronal dysfunction and cell death [172].

Cybrid Studies—Similar to AD, the cybrid model (Fig. 2) also supports a role for mitochondrial dysfunction in PD. Cybrids generated from PD subject mtDNA show reduced Complex I activity. These studies have used several different nuclear backgrounds, including neuroblastoma, teratocarcinoma and lung carcinoma cell lines [173–178]. Cybrids from PD subjects show increased ROS, increased mitochondrial calcium, increased mitochondrial ETC proton leak, and decreased mitochondrial membrane potential [173–175, 179]. PD cybrid cells have also been shown to exhibit increased oligomerization of α -synuclein [180] and develop Lewy bodies, a hallmark PD pathology [181]. These studies are consistent with mtDNA contributing to deficits observed in PD subjects.

Pre-Clinical PD Models—Mitochondria are the primary targets of the drug 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is used to induce PD in rodent and primate models [182, 183] and induces PD-like symptoms in humans [184]. MPTP is not accumulated in the brain but is converted by monoamine oxidase B to MPP⁺ which accumulates in dopaminergic neurons, a process which is required for neurotoxicity of MPTP [185]. It is postulated that the primary mechanism of action for MPTP involves an overproduction of free radicals and impaired mitochondrial function [186], and MPTP remains a popular animal model of PD. As mentioned, a role for mitochondria in PD is also supported by the integral function of mitochondrial monoamine oxidases on mediating dopamine turnover [187]. Monoamine oxidases have a high potential for hydrogen peroxide generation, which physiologically serves an important role in cell signaling [188]. However, hydrogen peroxide production can also be pathological; this messenger is widely considered

as the primary mechanism of toxicity of another PD-inducing chemical, 6-hydroxydopamine [189]. The substantia nigra is particularly vulnerable to hydrogen peroxide owing to its high iron content. In particular, this increases the propensity of hydrogen peroxide to undergo the Fenton reaction and produce hydroxyl radicals. Thus, an additional animal model of PD that remains highly utilized is the 6-OHDA model, which requires stereotaxic injection of this toxin into the desired location of the brain, which is generally either the striatum or fiber bundle projecting from the striatum to the substantia nigra (the nigrostriatal pathway).

PD Human Studies—Mitochondrial deficits, specifically involving Complex I, have been recognized to occur in human subjects with PD for decades [190–192], and the number of genes associated with PD that are involved in mitochondrial associated processes continues to grow [193]. A number of the different genetic variants that are implicated in autosomal recessive PD have been shown to directly impact mitochondrial function. The gene PTEN-induced putative kinase (PINK1), which is involved in autosomal recessive PD, has been suggested to serve as a regulator of mitophagy, and PINK1 mutations may interfere with the mitophagy process [194]. PINK1 has also been implicated in the transport of mitochondria within the cell [195]. A rare variant of the ATPase ATP13A2 has also been linked to PD [196]. ATP13A2 confers protection against rotenone-induced mitochondrial stress, potentially by recognizing signaling lipids, as an interaction with these lipids increases protection against mitochondrial stress [197]. Finally, another gene involved in autosomal recessive PD, PARK7, has been implicated in the antioxidant response and may play a role in mitochondrial function [198].

Interestingly, other variations in mitochondria occur that deem certain individuals seemingly resistant against PD. For instance, the mitochondrial haplogroup B5 has been shown to be protective against PD, and cybrids from this haplogroup are resistant to exposure to the mitochondrial toxin rotenone [199]. Antioxidants, which are widely recognized to protect mitochondria, have been shown to be altered in either level or activity in PD human subjects [200] and dopamine loss has been linked to increased levels of oxidized glutathione, suggesting a potential role of oxidative stress in PD [201].

Mitochondrial Medicine in PD

Due to the potential role of mitochondria in PD etiology, several drugs in development for PD treatment target mitochondrial function. Mitochondria-related processes that are current targets for intervention include exercise, antioxidants, and calcium homeostasis. Other metabolically active molecules which also affect mitochondria, including incretin hormones and PPAR- γ agonists, are examined in detail below.

Exercise

Evidence exists that exercise could be neuroprotective in preclinical models of PD. In a rat model of PD, treadmill exercise both reduced 6-OHDA mediated neurodegeneration and improved firing rates in striatal neurons [202]. Similarly, treadmill exercise was shown to increase markers of tyrosine hydroxylase in the SN and striatum following MPTP treatment in mice, indicating less MPTP-mediated cell loss, and exercise also improved motor balance and coordination measures in MPTP treated mice [203]. Some evidence suggests suppression of

microglial activation and decreased brain inflammation as a potential mechanism for exercise-mediated neuroprotection in the MPTP model [204] and other work suggests that exercise elicits positive effects on formation of dendritic spines after spine loss, potentially through neurotrophic factors or glutamate modulation [205]. More research into the mechanisms of exercise-mediated neuroprotection is warranted.

A number of clinical studies have evaluated the effect of exercise in PD. However, approaches differ between studies, including exercises such as walking, home-based training, and exercises targeted at improving characteristics of muscle, such as range-of-motion, strength, and muscle endurance. The modality of exercise and how the exercise is administered (group therapy, one-on-one or home based) is likely important to consider. One successful study of exercise in 60 PD subjects involved a type of therapy called LSVT BIG, which consisted of one-to-one exercise training involving whole-body movements and goal-directed activities of daily living. The BIG training, but not group exercise (Nordic walking) or educational instruction for domestic exercise, improved the UPDRS score in PD subjects [206]. A recent meta-analysis has found that resistance exercise training has a positive impact on muscle strength in PD, and that endurance exercise training can improve fitness in PD subjects. Although findings were inconsistent regarding the effect on disease severity (UPDRS score), no studies found that exercise therapy was detrimental [207]. Several studies of the effect of exercise in PD are ongoing or near completion. A randomized, single-blind pilot study to assess the effects of a home-based aerobic exercise program on cognitive outcomes and disease severity in PD subjects has been completed, but the results are not yet available (NCT01562496). Likewise, a trial assessing the effect of a 6 month stationary bike exercise program on disease severity (UPDRS) in PD subjects has been completed, but results are not yet available (NCT01741584). Finally, a study of high-intensity exercise training, which has been linked to improvements in metabolic outcomes, is currently ongoing in PD (NCT02593955).

Antioxidants

The key role of oxidative stress in modulating neurodegeneration in both MPTP and 6-OHDA animal models has made ROS an attractive drug target. Although the complex role of ROS as both secondary messengers and as damaging molecules, together with the difficulty of targeting antioxidants to the region where they are needed, has made ROS a difficult therapeutic focus [208]. Many drugs have been examined in animal and clinical models, with varying degrees of success.

Coenzyme Q10 and MitoQ—Coenzyme Q10 (CoQ10) has been investigated in several clinical trials in PD, the largest and most definitive being a phase 3 trial (NCT00740714) with a goal of evaluating the impact of CoQ10 on slowing clinical decline in early PD. This large randomized, placebo controlled study showed that CoQ10 was safe but elicited no evidence of clinical benefit compared to placebo [209] and was terminated when a futility criterion was reached. As discussed earlier, MitoQ is a mitochondrially-targeted antioxidant that contains CoQ10. MitoQ was recently evaluated in a double-blind, placebo-controlled Phase 2 clinical trial (NCT00329056). This study sought to examine the disease-modifying capability of MitoQ in PD patients. Subjects were treated with placebo, 40mg, or 80mg

MitoQ once daily for one year. However, neither treatment group differed in disease progression (UPDRS scale) compared to placebo [210].

N-acetyl-cysteine—N-acetyl-cysteine (NAC), which was discussed earlier in the context of AD, has also been tested in PD subjects. A small, phase I study (NCT01427517) using a single IV infusion of NAC showed a positive effect of NAC on both blood and brain glutathione levels [211]. A phase I/II trial is currently ongoing, which will evaluate the effect of one month of NAC supplementation in PD subjects (NCT01470027). An additional trial of oral NAC administration in PD subjects has been completed (NCT02212678), but the results are not yet available.

Mito-Apo/MPPE—The organic compound apocynin has anti-inflammatory properties that are linked to its role as an antioxidant. This compound has been modified to target mitochondria (Mito-Apo). Mito-Apo has been tested in preclinical PD models, where it was protective against loss of nigrostriatal neurons in response to MPTP [212]. Similarly, another drug that has shown efficacy in pre-clinical models is N-Methyl, N-propynyl-2-phenylethylamine (MPPE). This drug is an MAO-B inhibitor that protects against MPTP-induced neuronal damage. MPPE treatment has been shown to upregulate mitochondrial superoxide dismutase activity, and attenuates both damage to mitochondrial complex I and oxidative stress [213].

Pramipexole—Both the S(–) and R(+) forms of the drug pramipexole have been shown to target the mitochondria and exert antioxidant effects in cell models [214]. The S(–) enantiomer of pramipexole is currently used in human PD subjects due to its role as an agonist of the dopamine receptor, but the antioxidant properties of the R(+) enantiomer have led to investigations in both AD [215] and ALS [216]. These small, early studies have shown safety and tolerability but limited efficacy in humans.

ViNeuro—A randomized controlled trial of an additional drug with antioxidant properties, ViNeuro, in PD subjects has been completed. This drug enhances the antioxidant status of mitochondria, but the results of the trial are not yet available (clinicaltrials.gov ID NCT00517842).

Calcium Homeostasis

Calcium is also known to be an important secondary messenger in cell signaling cascades. Mitochondria are key players in calcium homeostasis, as they can accumulate and sequester calcium [217]. Calcium can, in turn, affect the expression of sirtuin proteins, which are involved in mitochondrial biogenesis. The sirtuin-2 inhibitor AK7 was found to be protective against MPTP-induced dopamine depletion and alpha-synuclein induced toxicity in animal and cell models [218]. This is in line with prior work showing that the same molecule was neuroprotective in a mouse model of Huntington's disease [219]. Sirtuin expression is affected by the accumulation of calcium in the mitochondrial matrix, and additional research has targeted calcium homeostasis itself in Parkinson's models [220]. Another drug, B355252, was shown to be neuroprotective against 6-OHDA in cellular models through a

mechanism involving both a decrease in ROS production and through a mitochondria-mediated mechanism involving calcium sequestration [221].

Calcium influx is controlled via L-type calcium channels, and the current through these channels in the substantia nigra is known to decrease with aging [222]. Multiple studies that have targeted L-type calcium channels with antagonists such as isradipine have shown neuroprotection in preclinical models [223–226]. Isradipine has recently been translated into human studies, and a recent Phase II clinical trial showed dose-dependent tolerability [227]. Although isradipine was not shown to be effective at significantly modulating the primary motor outcome measures, there was a trend toward benefit. A more definitive Phase III clinical trial of isradipine is currently underway. (clinicaltrials.gov ID NCT02168842).

Incretin Hormones

Ghrelin—Many hormones involved in glucose metabolism also modulate mitochondrial function. Ghrelin is a hormone that is released by the gastrointestinal tract following a meal. It has been linked to diverse processes, including satiety, insulin sensitivity, and even neuronal survival. In preclinical models, ghrelin has been shown to be protective against rotenone and MPTP. In cells, pre-treatment with ghrelin protected against rotenone-induced neurotoxicity, antagonizing cytochrome c release from mitochondria and activation of caspase-3 [228]. In animal models, pre-treatment of mice with ghrelin prior to MPTP administration resulted in attenuated striatal dopamine depletion [229]. This was potentially mediated through a mechanism involving mitochondrial membrane permeability, as ghrelin increased the Bcl-2 to Bax ratio and decreased caspase-3 activation [229]. Interestingly, recent research has shown that a particular isoform of ghrelin is important for its neuroprotective properties; acylated ghrelin, but not des-acylated ghrelin, is protective against MPTP in animal models [230].

Glucagon-Like-Peptide-1 (GLP-1)—An additional gut-released hormone, glucagon-like-peptide-1 (GLP-1) is important to both central and peripheral metabolism. As an incretin hormone, it can modulate pancreatic insulin release, and both ghrelin and GLP-1 can cross the blood brain barrier. A dual agonist of GLP-1 and glucose-dependent GIP called DA-JC1 was shown to reverse MPTP induced motor impairments in a PD animal model. This study showed an effect on the mitochondrial Bcl-2 to BAX ratio [231]. Interestingly, it has been shown in peripheral cell models, that a peptide consisting of the C-terminal domain of GLP-1, GLP1 (28–36), specifically targets mitochondria and inhibits stress-induced apoptosis [232]. The GLP-1 agonist Exendin-4 has shown promising results in PD subjects. In a single blinded human trial (ClinicalTrials.gov; NCT01174810) Exendin-4 was shown to be effective at improving motor function (MDS-UPDRS) compared to control subjects, who declined on the motor outcome [233]. A Phase II double-blind, placebo-controlled trial of this drug in PD subjects is currently underway (clinicaltrials.gov ID NCT01971242).

PPAR- γ Agonists

Thiazolidinediones, including rosiglitazone and pioglitazone, have been widely used clinically to treat Type 2 Diabetes. The mechanism of action of thiazolidinediones is activation of the proliferator-activated receptor gamma (PPAR- γ). PPAR γ expression has

been shown to protect mitochondria by maintaining membrane potential and preventing apoptosis in cell models [234]. These drugs have shown some promise in preventing PD, but only in certain populations. For instance, when individuals with diabetes who were taking these drugs were followed longitudinally (average 5.2 years), subjects who had taken the thiazolidinediones had a reduced incidence of PD [235]. However, a cohort study examining the use of thiazolidinediones and onset of PD found no delay in onset of PD [236]. Studies examining pioglitazone and rosiglitazone separately are discussed below.

Pioglitazone—Pioglitazone has been widely used clinically to treat Type 2 Diabetes due to its ability to lower blood glucose. In addition to its action of PPAR- γ , it has also been shown to bind to mitoNEET, a protein affiliated with the outer mitochondrial membrane, and it is argued that the mechanism of action for pioglitazone may extend beyond PPAR γ [237]. Due to its effects on cellular metabolism, pioglitazone was examined in preclinical PD models and shown to be neuroprotective [238, 239]. A Phase II randomized, controlled trial of pioglitazone has recently been completed (clinicaltrials.gov ID NCT01280123). This well-performed study in subjects with early stage PD showed that pioglitazone did not significantly modify PD progression vs. placebo [240] or alter biomarker levels [241]. Moreover, a primate study showed that while pioglitazone was effective at reducing dyskinesias, it reduced the effectiveness of L-DOPA therapy, calling the potential utility of this drug in more severe stages of PD into question [242].

Rosiglitazone—In cell models, pre-treatment of neuronal cells with rosiglitazone resulted in attenuation of MPTP toxicity, likely via a mechanism involving a reduction in ROS production, but potentially independent of PPAR γ [243]. In mouse models of progressive PD, rosiglitazone treatment was found to arrest neurodegeneration in the striatum and substantia nigra [244], as well as attenuate striatal dopamine depletion and microglia reactivity [245]. Despite these findings in preclinical models, no human studies of rosiglitazone in PD subjects have been published and none are ongoing.

Amyotrophic Lateral Sclerosis

ALS is a progressively fatal motor neuron disease. ALS is largely sporadic (90%), with approximately 10% of cases stemming from genetic mutations. Mutations in superoxide dismutase 1 (SOD1) account for approximately 20% of familial ALS cases [246]. Recently, mutations in TAR-DNA binding protein 43 (TDP43) have been implicated in both sporadic and familial forms of ALS [247–249]. Certain populations, such as the military, football, and soccer players have an increased risk for developing sporadic ALS [250–253]. While the underlying etiology is still unknown, in both familial and sporadic ALS, mitochondrial dysfunction is prevalent.

Cybrid Studies—As with studies in AD and PD, ALS cybrids recapitulate disease processes (Fig. 2). When cybrid cell lines were generated on an SH-SY5Y neuroblastoma nuclear background from platelets of sporadic ALS and control subjects, Complex I activity was significantly reduced and trends for reduced Complex III and IV activity were observed. ROS production was not altered in sporadic ALS cybrids, but there was a significant increase in the activity of antioxidant enzymes and reduced mitochondrial calcium

concentrations [254]. Another study used osteosarcoma cells as the nuclear background and reported no difference in gross mitochondrial respiration or ROS production in ALS cybrids [255]. However, this study did not examine specific ETC enzyme activity levels, and the nuclear background used could influence outcomes. A detailed review of cybrid studies can be found elsewhere [18].

Pre-Clinical ALS Models—SOD1 is principally a cytosolic protein. However, mutant forms of SOD1 localize to mitochondria, where it is hypothesized to disrupt mitochondrial function [256–258]. Mutant forms of SOD1 have been shown to induce a loss of mitochondrial membrane potential and mitochondrial swelling, with reductions in ATP production, disrupted calcium homeostasis, and loss of mitochondrial ETC enzyme activity [259–262]. Overall, mutations in SOD1 are strongly associated with mitochondrial and bioenergetic disruption.

Mutant SOD1 may inflict mitochondrial dysfunction by binding to and interacting with mitochondrial proteins. Mutant forms of SOD1 interact with BCL-2 in both mouse and human spinal cord [258]. The interaction between mutant forms of SOD1 and BCL-2 induces a “toxic conformation” in BCL-2, exposing the BH3 domain [257, 258]. The BH3 is known as the death domain; it is required for activation of apoptosis [263]. This “toxic” interaction could lead to mitochondrial dysfunction and cell death. Mutant forms of SOD1 interact with the mitochondrial voltage-dependent anion channel (VDAC), diminishing transport of ADP and lowering VDAC function [264]. Overall, mutant SOD1 proteins may promote mitochondrial dysfunction and bioenergetic failure by interacting with proteins which are central to mitochondrial function.

In vitro, mutant forms of SOD1 induce deficits in bioenergetic pathways, especially at the level of mitochondrial function. Overexpression of G93A mutant SOD1 in NSC34 motor neurons induces diminished ETC enzyme activity (Complex II and COX) and increases mitochondrial swelling [265]. Mutant forms of SOD1 alter redox balance (decrease GSH, increase GSSG), reduce mitochondrial ETC function (complexes I, II+III, and IV) and subsequently reduce ATP levels in the NSC34 motor neuron cell line [262]. SH-SY5Y cells transfected with G93A mutant SOD1 display loss of mitochondrial membrane potential, and increased cytosolic calcium concentrations [261]. In Neuro2A cells, localization of mutant SOD1 (both G93A and G85R) to mitochondria was associated with the release of cytochrome c and caspase-dependent apoptosis [266]. G93A mutant SOD1 expression in rat motor neurons induced deficits in mitochondrial fusion, retrograde axonal transport of mitochondria, reduced number of mitochondria, and loss of mitochondrial membrane potential [267]. These effects were specific to mutant SOD1; they were not observed with the expression of wild-type SOD1.

In vivo models of mutant SOD1 also show robust evidence of mitochondrial dysfunction. Mitochondrial dysfunction appears to occur prior to disease onset, such that G93A mutant SOD1 mice have decreased mitochondrial complex I activity at ~60 days of age, within the spinal cord [268]. Other studies have found that G93A mutant SOD1 leads to swollen and vacuolization of mitochondria within axons throughout the central nervous system (CNS). However, this particular study found no change in COX activity [269]. Other studies have

shown deficits in ETC enzyme activities within the ventral horn (complex I, II, and IV) and isolated mitochondria (COX) from spinal cord [270, 271]. Reductions in COX activity could be caused by a loss of cytochrome c association with the mitochondrial membrane. Further studies have depicted changes in mitochondrial morphology and axonal transport deficits within the spinal cord of G93A mutant SOD1 mice [272, 273]. Similar to *in vitro* studies, *in vivo* studies have found enzymatically active G93A mutant SOD1 within the intermembrane space of mitochondria. Mitochondria were dysfunctional in respiration, ETC enzyme activities, ATP synthesis and showed increased mitochondrial protein and lipid oxidation [274]. Mutant SOD1 expression in cells or animals induces numerous measures of mitochondrial and bioenergetic disruption.

TDP43 is a nuclear protein involved in gene transcription regulation, and similar to mutant SOD1, it forms insoluble aggregates within the cytoplasm [275, 276]. It was recently shown that both wild-type and mutant TDP43 (both Q331K and M337V) co-localize to mitochondria, induce changes in mitochondrial structure, and reduce mitochondrial number in primary mouse motor neurons [277]. NSC34 cells transfected with mutant TDP43 (both Q331K and M337V) had lower mitochondrial complex I activity, abnormal mitochondrial morphology, and a decrease in mitochondrial membrane potential [278]. Beyond *in vitro* results, *in vivo* models of mutant TDP43 also display mitochondrial dysfunction. In particular, mice which express A315T mutant TDP43 have mitochondrial transport deficits [273]. Overall, ALS familial mutations in TDP43 are associated with mitochondrial and bioenergetic deficits.

ALS Human Subjects—There is a large body of evidence for mitochondrial dysfunction in human ALS subjects. Mitochondrial abnormalities are observed in the CNS of sporadic ALS subjects. In dorsal root ganglion cells, ALS subjects have a higher number of mitochondrial inclusions, which appear to consist of aggregates within the cristae and intermembrane space [279]. Further studies showed abnormal mitochondrial morphology and aggregation of mitochondria in the anterior horn of the spinal cord [280]. COX activity was also reduced in sporadic ALS subject spinal cord tissue [281].

Functional deficits in spinal cord mitochondria might be due to changes in mtDNA. Spinal cord tissue had decreased levels of mtDNA and increased levels of mutated mtDNA in sporadic ALS subjects. This was in conjunction with lowered citrate synthase, Complex I + Complex III, and COX activities [282]. Motor cortex mitochondria had significantly higher levels of a common mtDNA deletion (mtDNA⁴⁹⁷⁷) when compared to the temporal cortex [283]. Higher levels of this deletion were also found in motor neurons from sporadic ALS subjects [284]. Muscle tissue offers further confirmation of mitochondrial dysfunction in ALS subjects. Muscle biopsy specimens show increased calcium levels and mitochondrial volume in motor nerve terminals [285]. ALS subjects have more COX negative muscle fibers (when compared to age-matched control subjects). In one such study ~46% of subjects had COX negative muscle fibers [286, 287]. Further, in subjects with COX negative muscle fibers an increase in mtDNA deletions and/or a loss of mtDNA were observed [287].

Systemic mitochondrial deficits are consistently reported in ALS. Skeletal muscle mitochondria from ALS subjects have decreased Complex I, COX, citrate synthase, and

succinate dehydrogenase activities and changes were also observed in fibroblasts [286–290]. However, “early” sporadic ALS subjects failed to show a change in basal, maximum, Complex I-linked, Complex II-linked, or COX-linked mitochondrial respiration from muscle biopsies [291]. As observed in the motor cortex, muscle mitochondria from ALS subjects have higher levels of the common mtDNA deletion (mtDNA⁴⁹⁷⁷) [292]. Mitochondrial morphology is altered in muscle biopsies [293–295]. Changes are also observed in lymphocytes. While basal mitochondrial respiration was not changed, when respiration was stimulated with an uncoupler, the response was depressed in ALS subjects. This suggests lymphocytes from ALS subjects have decreased mitochondrial spare respiratory capacity [296]. Platelet mitochondria from ALS subjects have depolarized membrane potential and a higher rate of apoptosis [297]. Further studies found a correlation between protein carbonyl concentration in blood and clinical symptom onset in sporadic ALS subjects, including higher markers of lipid peroxidation in plasma, but no change in plasma antioxidant levels [298]. Changes are also evident in erythrocytes from sporadic ALS subjects. Increased lipid peroxidation, decreased antioxidant enzyme activities (glutathione reductase), decreased activity of glucose 6-phosphate dehydrogenase, and reduced levels of GSH were observed [299]. The severity of these changes correlates with disease progression and is supported in other studies [299, 300]. Oxidative DNA damage is observed at a higher rate in ALS subjects, from urine, CSF, and plasma samples [301].

Mitochondrial biogenesis pathways are also affected in ALS subjects. Within the spinal cord and motor cortex, the expression of nuclear respiratory factors 1 and 2 (NRF1 and NRF2), and mitochondrial transcription factor A (TFAM) are reduced. The motor cortex also had lower expression of PGC1 α . Some of these changes were also observed in muscle, where PGC1 α , NRF1, and NRF2 expression were reduced [302]. Further analysis of spinal cord revealed reduced PGC1 α immunohistochemical staining in ALS spinal cord compared to control [287].

In summary, evidence of mitochondrial and bioenergetic disruption in ALS exists in both preclinical ALS models and familial and sporadic human ALS subjects. Studies have depicted mitochondrial abnormalities in cell culture, animal models, and human subjects. ETC activities, mitochondrial morphology, and ROS production appear to be aberrant. While the underlying cause for these observations is not clear, evidence of mtDNA deletions and interactions between proteins implicated in familial ALS and mitochondria are prominent. Further detailed information outlining mitochondrial dysfunction in ALS can be found in an eloquent review by Muyderman and Chen [303].

MITOCHONDRIAL MEDICINE IN ALS

Lifestyle Alterations

Diet

Ketogenic Diet: The ketogenic diet showed positive effects in the G93A mutant SOD1 mouse model. Mice that received the ketogenic diet, beginning at 50 days of age, had higher levels of serum ketone bodies (β -hydroxybutyrate and acetoacetate), maintained body weight, and displayed sustained rotarod performance when compared to G93A mutant

SOD1 mice maintained on normal chow [304]. While G93A mutant SOD1 mice maintained on a ketogenic diet did not have the same number of motor neurons when compared to wild-type mice, they did have significantly more motor neurons than G93A mutant SOD1 mice maintained on normal chow. The ketone body β -hydroxybutyrate showed positive effects on spinal cord mitochondria isolated from G93A mutant SOD1 mice. β -hydroxybutyrate increased the rate of ATP synthesis in un-stressed mitochondria and mitochondria treated with rotenone. Further, β -hydroxybutyrate protected spinal cord motor neurons from rotenone induced cell death [304]. A pilot clinical study is reported through clinicaltrials.gov (NCT01016522), however the study is listed as terminated with no data or publications reported. Beyond this, the ketogenic diet in ALS subjects has not been extensively tested.

Medium Chain Triglycerides: The medium chain triglyceride caprylic triglyceride can cross the blood brain barrier and has shown promise in animal models. In G93A mutant SOD1 mice fed a diet enriched for caprylic triglyceride, enhanced motor performance (shown as latency to fall) was observed beginning at 50 days of age. Although mice did not show increased longevity, there was a significant preservation of motor neuron number within the spinal cord. The caprylic triglyceride enriched diet *also* increased plasma ketone levels in post-symptomatic G93A mutant SOD1 mice. Mitochondria isolated from spinal cord of G93A mutant SOD1 mice fed a caprylic triglyceride enriched diet, had an increase in basal respiration and maximum respiration compared control mice [305]. Currently, a pilot clinical study is enrolling to test caprylic triglyceride in ALS subjects (clinicaltrials.gov ID NCT02716662).

The Deana Protocol Supplement: Other dietary modulations may be beneficial in ALS. One study compared the effects of the ketogenic diet to the administration of a supplement known as the Deana protocol. The Deana protocol is a supplement which consists of, arginine alpha-ketoglutarate, gamma-aminobutyric acid (GABA), ubiquinol (Coenzyme Q10; CoQ10), and medium chain triglycerides. This therapy was administered beginning at ten weeks of age in the G93A mutant SOD1 mouse model. Mice which received a standard diet with the Deana Protocol supplement had improved neurological score, performed better on the rotarod and Paw Grip Endurance Test (PaGE) tests, and increased longevity. Mice that received a ketogenic diet *alone* did not show increased longevity but did improve on rotarod, grip strength, and PaGE testing. Mice maintained on the ketogenic diet in conjunction with the Deana protocol supplement had a significant extension in life span [306]. The ketogenic diet, ketogenic diet with Deana protocol supplement, and standard diet with Deana protocol supplement groups all had increased brown adipose tissue, but no change in body weight.

Exercise—Exercise has shown positive outcomes in pre-clinical ALS models. Exercise prior to disease onset in the G93A mutant SOD1 mouse model extended life span by approximately ten days (in male mice only, [307]). In a separate study, moderate and high-intensity exercise were compared in the G93A mutant SOD1 mouse model. Moderate exercise delayed disease onset by approximately seven days, while high-intensity exercise increased the rate of motor decline, as shown by rotarod performance [308]. The hastened motor decline observed with high-intensity exercise has been observed in other studies

[309]. However, both moderate and high intensity exercise appeared to slow the loss of motor neurons in the G93A mutant SOD1 mice. The effects of exercise in the G93A mutant SOD1 mouse model appear to be “dose” dependent. Kaspar *et al.* found that 6 hours of exercise per week extended life-span and rotarod performance in G93A mutant SOD1 mice, and a synergistic effect was observed between exercise and insulin growth factor 1 (IGF1) overexpression [310].

A limited number of clinical trials support exercise as a therapy for ALS. In a small, randomized trial, resistance exercise improved patient outcomes when measured using the ALS Functional Rating Scale (ALSFRS-R) and upper and lower extremity subscale scores [311]. Another randomized controlled trial of exercise in ALS showed no benefits to survival or quality of life, but did report a positive outcome for the ALSFRS-R score [312]. A recent review of exercise trials for ALS has concluded more studies are needed to determine the risks and benefits of exercise in ALS [313].

Rasagaline—N-Propargyl-1(R)-aminoindan, or rasagaline, is a pharmaceutical which inhibits MAO-B. MAO-B is an outer mitochondrial membrane protein. The expression of MAO-B is increased in the brain of AD and PD subjects. MAO-B catalyzes the oxidative deamination of monamine neurotransmitters such as dopamine and serotonin, a process which produces hydrogen peroxide [314]. For this reason, rasagaline was found to be protective in PD. However, rasagaline was also found to activate antioxidant and anti-apoptotic mechanisms. *In vitro* studies have shown rasagaline improves mitochondrial function. Rasagaline increases the expression of the anti-apoptotic protein Bcl-2 and Bcl-X_L in neuron-like cells [315]. Further, rasagaline is protective in cell culture models against several insults, including oxidative stress and serum starvation [315–319]. *In vivo*, rasagaline increased survival and running wheel performance (in a dose-dependent manner) in the G93A mutant SOD1 mouse model. In addition to these findings, rasagaline in combination with riluzole displayed additive effects [320].

Rasagaline has shown positive effects in clinical trials. In white blood cells, mitochondrial membrane potential was increased. In whole blood, the Bcl-2/Bax ratio was increased. Oxidative stress, measured by an Oxygen Radical Absorbance Capacity (ORAC) assay, was reduced. However, this study failed to find changes in the ALSFRS-R score [321]. Several larger clinical trials are in progress, which include functional and biomarker outcome measurements (clinicaltrials.gov IDs NCT01879241, NCT01786603).

Antioxidants

Vitamin E: A number of studies have tested the effects of antioxidant therapies in ALS. Early studies focused on the effects of insoluble antioxidants such as Vitamin E. Pre-clinical testing of Vitamin E in the G93A mutant SOD1 mouse model revealed delayed disease onset and progression but did not extend survival [322]. Clinically, vitamin E has been associated with decreased ALS risk, as well as increased antioxidant activity and decreased oxidative stress markers in plasma [323–325]. However, clinical trials have not shown a clear benefit of vitamin E in ALS subjects. One trial assessed outcomes after one year of daily vitamin E. No effect on the primary outcome (rate of deterioration – Norris limb scale) was observed,

although a secondary outcome of disease progression did show benefit, and serum oxidative stress markers were decreased in the treatment group [325]. An observational study found no beneficial effect of Vitamin E in ALS subjects [326]. In a large, double-blind, placebo-controlled study the use of high dose Vitamin E did not show significant effects on survival or rate of deterioration, although larger studies may be needed [327].

Coenzyme Q10: In pre-clinical studies, adding CoQ10 to the chow increased the probability of survival in the G93A mutant SOD1 mouse model [328]. However, CoQ10 has thus far failed to translate clinically. In a phase II trial no efficacy on outcomes were observed [329]. As discussed in the AD therapeutics section, a novel mitochondrial targeted antioxidant which contains CoQ10 (MitoQ) is being developed for clinical testing.

SS Peptide Antioxidants: SS peptide antioxidants have been evaluated for efficacy in *in vivo* models of ALS. In particular, SS31 increased survival and motor performance in the G93A mutant SOD1 mouse model. Indices of oxidative stress were reduced in the lumbar spinal cord, and cell loss was abrogated in G93A SOD1 mice which received SS31 [330]. However, no human clinical trials have been reported for SS peptide antioxidants.

Olesoxime: Olesoxime is a lipophilic, cholesterol-like compound which is known to modulate the mitochondrial transition pore. Olesoxime binds to mitochondrial localized VDAC and is protective in several models of cell death and animal models of ALS. These studies and the pharmacological mechanism of Olesoxime are reviewed elsewhere [331]. However, a more recent phase II/III clinical trial failed to show beneficial effects in ALS subjects [332].

Glutathione: Reduced GSH is an endogenous soluble antioxidant with cellular compartmentalization within the cytoplasm, nucleus, and mitochondria. GSH is important for maintaining mitochondrial function [158]. The levels of GSH, particularly in the mitochondria are reduced *in vitro* when G93A mutant SOD1 is overexpressed in mice [333]. Genetic ablation of a key GSH synthesis enzyme worsens the progression of ALS symptoms in G93A mice [334]. In that model, reductions in spinal cord GSH concentration are observed and correlate with expression of apoptotic markers in motor neurons [335]. A whey protein supplement, Immunocal® has been tested in preclinical model. This supplement boosts GSH levels by providing the glutathione precursor, cysteine. In the G93A mice, Immunocal® delayed disease onset, improved rotarod performance, but failed to extend survival. While Immunocal® preserved whole blood and whole spinal cord levels of GSH in G93A mice, it failed to preserve mitochondrial GSH levels [336]. This suggests that targeting antioxidants within the mitochondria is important for disease outcomes. Overall, several key issues will need to be addressed in future clinical studies, such as GSH is not cell permeable, and targeting GSH to the mitochondria. This may explain the lack of effect of reduced GSH on disease progression in an open label clinical study [337].

Dexpramipexole: Dexpramipexole is the R(+) enantiomer of pramipexole and a dopamine receptor antagonist. Dexpramipexole has displayed protective effects *in vitro*, where it mitigated ROS, improved mitochondrial function and prevented apoptosis [214, 338]. However, *in vivo*, dexpramipexole failed to show beneficial outcomes in either G93A mutant

SOD1 mice or rat cortical neurons overexpressing M337V mutant TDP43 [339]. These pre-clinical studies were published following a positive phase II, but failed phase III clinical trial of dexamipexole in ALS subjects [340, 341].

A meta-analysis of antioxidant therapies in ALS found no beneficial effect of antioxidants within the studies examined (9 studies included in the analysis). These antioxidants included vitamin E, acetylcysteine, or a combination therapy composed of L-methionine, vitamin E, and selenium [342]. This study discussed very important issues with clinical trials of antioxidants in ALS. These issues include a low number of participants and poor study design [343, 344].

THE FUTURE OF MITOCHONDRIAL MEDICINE

A major issue for therapeutic development in neurodegenerative diseases is the disparity between pre-clinical and clinical study outcomes. Many potential therapeutics that show positive effects in cell and animal models fail to be effective in human subjects—it is estimated that more than 80% of therapeutics fail clinical testing [250, 345–348].

Hurdles to Progress

Pre-Clinical Studies—Explanations for the lack of translational success disparity vary, but likely involve a combination of factors. First, there are innate differences between humans and animal model species. For example, mice have higher uric acid levels among other endogenous antioxidant concentrations and properties [250]. Second, many animal models for neurodegenerative research are based on familial forms of the disease. While these models have been pivotal in understanding disease mechanism, they may not faithfully replicate sporadic forms of disease or perhaps emulate the asymptomatic rather than the symptomatic phase of the disease [250, 349]. Many studies in animal models use animals of the same sex, and it is also possible that sex differences in human studies may impact results. Moreover, some predilections estimate that animal model studies over-predict potential success in human subjects by approximately 30%, and this might be caused by failure to publish negative results [345, 346]. Beyond not publishing negative results, others suggest animal studies are poorly designed [347, 348]. For instance, the ALS Therapy Development Institute re-tested over 100 compounds using more rigorous experimental design and found very conflicting results from published data [348]. Many issues that need to be reliably addressed in pre-clinical studies include gender matching between experimental groups and ensuring that transgenes (such as G93A SOD1 or mutant APP) are inherited in a consistent manner in an animal colony [347, 348]. More stringent study design and testing across multiple preclinical models may increase the likelihood of translational success.

Human Studies—Additional reasons that therapies may fail to translate may be linked to the design of clinical trials. In several instances, it has been suggested that the treatment was initiated too late in the course of the disease to impact the designated outcome measures. In addition, mitochondrial medicine for neurodegenerative disorders faces a somewhat unique issue related to target engagement. This is because in addition to identifying compounds which penetrate the blood brain barrier, drugs also must reach the mitochondria, which is

often difficult. These factors may partially explain conflicting results of clinical trials, such as those examining the effect of various antioxidants [350].

It should be noted that most of these hurdles are not unique to mitochondrial medicine, but are a snapshot of the challenges facing neurotherapeutic drug development. In recent years, approvals for new neurological drugs have not kept pace with research funding; in fact, less than 10% of neurological drugs have a likelihood of approval from phase I [351, 352]. However, increasing translation in the neurosciences has become an area of focus [353].

Future Directions—Evidence here suggests that drugs and interventions that target mitochondria are being actively investigated in the most common neurodegenerative disorders. The bulk of investigations are being performed in pre-clinical models, but some drug candidates, such as Isradipine for Parkinson's disease, have progressed from preclinical work all the way to ongoing Phase III clinical trials. Novel approaches including targeting antioxidants to the mitochondrial matrix using nanoparticles, and lipophilic cations are promising endeavors. Further, stem cell transplantation therapies may mitigate mitochondrial dysfunction. For example, in APP/PS1 AD mice that received neuronal stem cell transplantation, an increase in mitochondrial number, improved mitochondrial fission/fusion dynamics, and improved cognitive decline were reported [354]. Overall, the vast evidence for a role of mitochondria in neurodegenerative disorders underscores the importance of continued investigation into therapeutics that modulate mitochondrial dysfunction for the treatment of neurodegenerative disorders.

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LIST OF ABBREVIATIONS

6-OHDA	6-hydroxy dopamine
AD	Alzheimer's disease
ADAS-Cog	The Alzheimer's disease assessment scale. – cognition
ADCS-ADL	Alzheimer's disease co-operative study - activities of daily living inventory
ADL/IADL	Activities of daily living/instrumental activities of daily living
ADP	Adenosine diphosphate
AICD	Amyloid precursor protein intracellular domain
ALS	Amyotrophic lateral sclerosis

ALSFRS-R	ALS functional rating score
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
Aβ	Amyloid beta
BACE	β -secretase
BAX	Bcl-2 associated protein X
BCL-2	B-cell lymphoma 2
BCL-XL	B-cell lymphoma extra-large
BDNF	Brain derived neurotrophic factor
<i>C. elegans</i>	<i>Caenorhabditis elegans</i>
CCL11	C-C motif chemokine 11
CDR	Clinical dementia rating
ChAT	Choline acetyltransferase
CNS	Central nervous system
CoQ10	Coenzyme Q10
COX	Complex IV or cytochrome oxidase
CSF	Cerebral spinal fluid
Cybrid	Cytoplasmic hybrid
DAMP	Damage associate molecular pattern
DHA	Docosaehaenoic acid
DNA	Deoxyribonucleic acid
ETC	Electron transport chain
FADH₂	Flavin adenine dinucleotide
FDG-PET	Fludeoxyglucose (¹⁸ F) positron emission tomography
GABA	Gamma-aminobutyric acid (γ -aminobutyric acid)
GDS	Geriatric depression scale
GIP	Gastric inhibitory peptide
GLP-1	Glucagon like peptide 1

GSH	Reduce glutathione
GSSG	Oxidized glutathione
IGF1	Insulin like growth factor 1
K_m	Michaelis constant
L-DOPA	Levodopa or L-3,4-dihydroxyphenylalanine
MAOB	Monamine oxidase B
MCI	Mild-cognitive impairment
MDA	Malondialdehyde
MDRS	Mattis dementia rating scale
MDS-UPDRS	MDS unified Parkinson's disease rating scale
MitoQ	Mitochondrial targeted coenzyme Q10 or Phosphonium, [10-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)decyl]triphenyl-, methanesulfonate
MMSE	Mini-mental state exam
MPPE	N-Methyl, N-propynyl-2-phenylethylamine
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mtDNA	Mitochondrial DNA
mtDNA⁴⁹⁷⁷	Common deletion of mitochondrial DNA at bp 4977
NAC	N-acetyl cysteine
NADH	Nicotinamide adenine dinucleotide
NRF1	Nuclear respiratory factor 1
NRF2	Nuclear respiratory factor 2
OAA	Oxaloacetate
ORAC	Oxygen radical absorbance capacity
PaGE	Paw-grip endurance test
PD	Parkinson's disease
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PRC	PGC1 related coactivator

ROS	Reactive oxygen species
SIRT1	Sirtuin 1
SOD1	Cu/Zn superoxide dismutase
SS-peptide	Szeto-Schiller Peptide
TBARS	Thiobarbituric acid reactive substances
TCA	Citric acid cycle
TDP43	TAR DNA-binding protein 43
TFAM	Mitochondrial transcription factor A
VDAC	Voltage-dependent anion channel
VEGFA	Vascular endothelial growth factor A
VO₂ Max	Maximal oxygen consumption or maximal aerobic capacity
ρ0	Rho zero- a cell lacking mtDNA

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Fig. (1).

Bioenergetic Pathways and neurodegenerative diseases. Glucose is catabolized in the cytoplasm to pyruvate through the glycolytic pathway. Pyruvate is used as a substrate in the TCA cycle within the mitochondria. The TCA cycle generates the intermediates NADH and FADH₂. These intermediates enter the ETC at complex I (NADH) and II (FADH₂). Complex IV (or COX) directly provides electrons to Complex V, driving ATP production. The ETC complexes are shown in red. Bioenergetic molecules are shown in blue. Glucose utilization is decreased in AD, PD, and ALS subjects. Within the ETC, Complex I function is reduced in PD subjects, while Complex IV (or COX) function is reduced in AD subjects. ALS subjects show deficits in several ETC complexes, depending on the tissue examined.

Fig. (2). Schematic depicting the generation of cybrid cell lines. A “normal cell” is depleted of mtDNA (shown in blue) through treatment with ethidium bromide. This cell is now a $\rho 0$ cell, lacking mtDNA but still containing nuclear DNA encoded mitochondrial components (shown in red). Fusion of the $\rho 0$ cell with a donor cytoplasm (such as a platelet) allows transfer of donor mtDNA (shown in green) to the $\rho 0$ cell, generating a cytoplasmic hybrid or cybrid.

Fig. (3). Schematic depicting the mechanism of mitochondria centered therapeutics

A. Antioxidants and PPAR γ agonists inhibit or scavenge ROS production. B. Diet and Exercise activate mitochondrial biogenesis through the activation of SIRT1 and/or PGC1 α . C. Rasagaline, PPAR γ agonists, and metabolic hormones inhibit the opening of the mitochondrial permeability transition pore (MPTP) and release of cytochrome c. These therapeutics also increase Bcl-2 expression and lower Bax expression. Bax maybe a part of the MPTP. The mechanisms listed here are not all encompassing.