

MINI-SYMPOSIUM: ENERGY DEMAND AND ENERGY SUPPLY IN ALZHEIMER'S DISEASE

Alzheimer's Disease: From Mitochondrial Perturbations to Mitochondrial MedicineSusana Cardoso^{1,2}; Cristina Carvalho^{1,2}; Sónia C. Correia^{1,2}; Raquel M. Seica^{3,4}; Paula I. Moreira^{1,3}¹ CNC—Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.² Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal.³ Laboratory of Physiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal.⁴ IBILI-Institute for Biomedical Imaging and Life Sciences, Faculty of Medicine, University of Coimbra, Coimbra, Portugal.**Keywords**

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Abstract

Age-related neurodegenerative diseases such as Alzheimer's disease (AD) are distressing conditions causing countless levels of suffering for which treatment is often insufficient or inexistent. Considered to be the most common cause of dementia and an incurable, progressive neurodegenerative disorder, the intricate pathogenic mechanisms of AD continue to be revealed and, consequently, an effective treatment needs to be developed. Among the diverse hypothesis that have been proposed to explain AD pathogenesis, the one concerning mitochondrial dysfunction has raised as one of the most discussed with an actual acceptance in the field. It posits that manipulating mitochondrial function and understanding the deficits that result in mitochondrial injury may help to control and/or limit the development of AD. To achieve such goal, the concept of mitochondrial medicine places itself as a promising gathering of strategies to directly manage the major insidious disturbances of mitochondrial homeostasis as well as attempts to directly or indirectly manage its consequences in the context of AD. The aim of this review is to summarize the evolution that occurred from the establishment of mitochondrial homeostasis perturbation as masterpieces in AD pathogenesis up until the development of mitochondrial medicine. Following a brief glimpse in the past and current hypothesis regarding the triad of aging, mitochondria and AD, this manuscript will address the major mechanisms currently believed to participate in above mentioned events. Both pharmacological and lifestyle interventions will also be reviewed as AD-related mitochondrial therapeutics.

INTRODUCTION

It has been a long road since the first case of Alzheimer's disease (AD) was observed by Alois Alzheimer in 1901. Back then, the young physician observed a patient complaining from deteriorating memory, even of recent events, disorientation, decreasing speech abilities and lack of judgment of the different surrounding situations (132). Later, in patient post-mortem brain investigation, it was visible the thinning of the cerebral cortex together with two characteristic lesions, neurofibrillary tangles (NFTs) and extracellular deposits that would be later named senile plaques (154).

Beginning with memory loss of recent events (short-term memory impairment) that proceed depriving the patients of their sense of self, AD represents about 50%–70% of all dementia cases affecting 4%–8% of the elderly population worldwide (4). Due to its age-related incidence, as population ages, the prevalence of AD is expected to be more than triple by 2050, reaching over 115 million, what will represent a great threat to older individuals and their families, becoming a serious social problem with increasing longevity (171). Often diagnosed in people aged 65 (>95%) and older, the

disease is typically referred to as late-onset AD, occurring due to a sporadic component; in contrast to early-onset AD, where initial symptoms can be observed between 30 and 65 years of age and occurs due to a familiar genetic cause involving mutations in the amyloid- β protein precursor (APP) and presenilins 1 and 2 (PS1 and PS2) genes (69). Clinically, AD is characterized by a progressive memory and cognitive impairment caused by the extensive death of neurons. Starting in the entorhinal cortex and hippocampus and later proceeding to other parts of the brain cortex and subcortical gray matter, neuronal loss gradually compromises brain health of the patients, culminating in the complete need for care within several years after clinical diagnosis (69). The main pathological hallmarks found in affected parts of the AD brain concerns two neurodegenerative processes: accumulation of amyloid β -peptide (A β), leading to the presence of extracellular A β deposits; and neurofibrillary degeneration, corresponding to the formation of intracellular deposits of the microtubule-associated protein tau as NFTs (185, 192). Of note, AD has an insidious onset and it has been estimated that neurodegeneration begins 20–30 years before the clinical manifestations become evident (139). However, and despite the

progresses in basic and clinical research that occurred after the discovery of Alois Alzheimer, until now AD still lacks disease-modifying treatments that can influence the underlying pathogenic phenotypes of the disease (65). A condition that is not facilitated by the fact that AD is largely idiopathic, with many proposed hypotheses to explain its pathophysiology. In this regard, one of the most debated theory implies mitochondrial dysfunction and oxidative stress as early events in AD development and potential therapeutic targets (207).

As postulated in an impressive body of evidence, neuronal survival critically depends on the integrity and functionality of mitochondria. In order to achieve the well-being of such vital organelles, a hierarchical system of cellular surveillance mechanisms protects mitochondria against stress, monitors mitochondrial damage and ensures the selective removal of dysfunctional mitochondrial proteins or organelles (181). However, in situations that a failure of this system occurs, damaged mitochondria will emerge as a central piece in cell energy deficiency and disruption of cell function, which will eventually compromise neuronal health leading to neurodegeneration (20). In this scenario, with its proved involvement in aging and in the early pathogenesis of sporadic AD, mitochondria represent attractive targets for treatment strategies. As so, as the incessant search for new compounds and therapeutic tools for AD continues, nowadays it can include pharmacological and lifestyle interventions, both with the rational of directly manage mitochondrial dysfunction as well as attempts to directly or indirectly manage its consequences (205). In this manuscript, we will start with a brief contextualization of the past and current hypothesis regarding the triad of aging, mitochondria and AD. Later we will summarize key aspects of mitochondrial abnormalities in AD and review mitochondrial medicine approaches to target and/or manipulate such disturbances in order to re-establish mitochondrial homeostasis.

INSIGHTS INTO MITOCHONDRIAL HOMEOSTASIS PERTURBATION IN AD

It has been some years since mitochondrial structural and functional perturbations were noted in AD and it was agreed that mitochondria from AD subjects differ from those of age-matched, non-demented subjects (206). Beginning in the 70s, electron microscopy images revealed an abnormal mitochondria morphology in the brains of AD subjects (86), a condition that was corroborated years later and postulated to perhaps even precede dendritic degeneration (186). Later, systemic alterations in calcium homeostasis in AD patient fibroblasts suggested for a systemic bioenergetics defect (165). Also in the 80s, a possible major role for energy metabolism and mitochondria defects in AD was proposed, when it was noted that AD brains possess reduced activities of several mitochondria-localized enzymes, including α -ketoglutarate dehydrogenase (α -KGDH) and pyruvate dehydrogenase (PDH) enzymatic complexes, suggesting AD as a disease of perturbed brain energy metabolism (17, 63, 64, 199). Nowadays it is widely accepted that mitochondrial dysfunctions involved in AD pathophysiology may include disturbances in oxidative phosphorylation (OXPHOS), and impaired energy metabolism as well as excess generation of reactive oxygen species (ROS), altered mitochondrial biogenesis, transport and dynamics (82). The next subsections briefly discuss the interrelation of aging, mitochondria and AD and,

then, an update of the body of research that describes the pathophysiological effects of mitochondrial dysfunctions in AD will be presented.

The aging process, mitochondria and AD: A glimpse into the main hypothesis

Aging, an unavoidable biological process, is characterized by a general decline in physiological function that leads to morbidity and mortality (222). Due to the exponential increase of world aging population, the increase in life expectancy and, the consequent social burden of health care and pharmacoeconomic systems, the study of aging has become one of the major fields of basic and clinical research (193). As featured in a compelling body of proof, some aging paradigms place mitochondrial DNA (mtDNA) mutations and oxidative damage as main contributors to the aging process (76). As stated in the "free radical theory" of aging, proposed by Harman in the 50s (74), the gradual accumulation of damage within cellular macromolecules (eg, nuclear and mitochondrial DNA, lipids and proteins) with age could be attributed to deleterious effects of free radicals produced by aerobic metabolism. Thus, although mitochondria are responsible for energy production, they also have another, less beneficial legacy in the cell, the continuous production of potentially harmful ROS, which has become an essential focus of aging research (12). Using animal models such as the senescence-accelerated mice (SAM) strain, researchers could explore the mechanisms of the age-related mitochondrial decline (98, 196, 233). Among the results observed, behavioral studies showed that learning and memory deficits already started as early as 6 months and worsened with aging in SAMP8 (accelerated senescence-prone 8) mice (158). Further, it was demonstrated that aging is also connected with an unbalance of the protective antioxidant machinery inside mitochondria. For instance, age-related changes in levels of antioxidant enzymes, such as copper/zinc superoxide dismutase (Cu/Zn-SOD) and manganese SOD (Mn-SOD), have been found in liver and cortex of SAMP8 mice when compared with age-matched SAMR1 (accelerated senescence-resistant 1) mice, supporting increased oxidative stress as a key mechanism involved in the aging process (97). Of note, besides the progressive mitochondrial decline and increased oxidative stress, this mice model also presented tau hyperphosphorylation at an early age (3, 204) and, an age-related increase in mRNA and protein levels of APP. In particular, the cleavage product A β was significantly increased at 9 months in SAMP8 and amyloid plaques started to form at around 16 months of age (143, 211). So, the understanding that mitochondrial dysfunction can act as a prominent and early, chronic oxidative stress-associated event that contributes to synaptic abnormalities in aging and, ultimately, increased susceptibility to age-related disorders including AD, conducted Harman to rename his previous hypothesis to "mitochondrial theory of aging" (75).

By definition, AD is an age-related progressive neurodegenerative disorder mainly affecting elderly individuals and, as previously suggested, the manifest common end point of brain aging and AD is the impairment of memory and cognition, and at the molecular and cellular level the similarity is very striking between these two conditions (206). Nonetheless, one question that remains without an absolute answer is why the aging brain progresses to AD with extensive neurodegeneration in some cases but not in others. The first clues,

that are in the basis of the “amyloid cascade hypothesis,” came with the identification of familial AD mutations in APP, PS1 and PS2 genes, which have given a big lift to the hypothesis of proteotoxic mechanisms mediated by the oligomers of A β in explaining the neurodegeneration of the sporadic AD (71, 73). Briefly, the amyloid cascade hypothesis, which has prevailed for more than 20 years in the AD field, speculates that in the common, late-onset AD variants, mutations or polymorphisms in genes that regulate A β production or removal directly control A β levels, oligomers formation and AD itself (206). From this point, AD-related research uncovered a plethora of mechanistic pathways triggered by A β oligomers that culminate in the apoptotic or autophagic death of neurons (72). Despite that, most of the investigational drugs available targeting APP and A β have consistently failed (56). Also, new evidence revealed some inconsistencies in the amyloid hypothesis, namely that the amount of A β deposition in the brain of sporadic AD patients does not always correlate with cognitive impairment and, inversely, A β deposition can also be found in cognitively normal individuals (32, 78, 144). In fact, the underlying process that leads to familial AD appears to be distinct from that leading to late-onset AD and so, at this stage, another hypothesis has been raised. Knowing that APP processing is a highly regulated event directly affected by bioenergetics metabolism and, having in mind the previous assumption that with advancing age mitochondrial function changes, investigators hypothesized that the accumulation of A β could be a consequence of mitochondria damage during aging rather than the cause of the neuropathological cascade in sporadic AD (208). As so, all of those concepts were brought together in the “mitochondrial cascade hypothesis” (209). In brief, this hypothesis assumes that inheritance defines an individual's baseline mitochondrial function that is affected by environmental factors determining the speed at which age-associated mitochondrial changes appear, and along with this influence, mitochondria may accumulate damage resulting in both the symptoms and neuropathology found in AD (208). Even though this hypothesis is still actual with several arguments in favor, as investigation proceeds, a new neuroenergetics perspective has been recently postulated in AD field, the “Inverse Warburg hypothesis” (45). Proposed by Demetrius and Simon (46), this hypothesis tries to explain the pathogenesis of sporadic AD cases, and postulate that AD is a metabolic disease initiated by an age-related mitochondrial deficit (44–46). Concisely, this hypothesis is based on the mitochondrial cascade hypothesis and implicates energy and age, as the critical elements in the origin of neurodegenerative diseases. In this regard, it posits that the primary cause of sporadic forms of AD is an age-induced energy deficit in the mitochondrial activity of neurons, and the increased ROS production and oxidative stress that surpasses a threshold after the failure of compensatory mechanism (the upregulation of oxidative phosphorylation), this will trigger the amyloidogenic pathway leading to A β accumulation, death of susceptible neurons and dementia (45, 66). Of note, it cannot be neglected that similar to genetic risk factors, environmental and lifestyle risk factors for AD can also be implicated in the acceleration of aging, morbidity and mortality (77).

Overall, a never-ending number of studies emphasize that mitochondrial dysfunction, and its consequences, have a primary role in the neurodegenerative processes and, may indeed represent the missing link between aging and sporadic AD. Thus, presently, the goal is to find alternative approaches and/or future directions in understanding the neurodegenerative process from the known

mechanisms of cellular aging that may eventually provide a rationale to new therapeutic possibilities in confronting the brain deficits of aging and AD.

Maternally inherited, mitochondria are considered the most complex and metabolically active organelles in the cell, being often denominated as the “powerhouses” of cells (11). Mitochondria play a paramount function in cell survival and death by responding to physiological and environmental signs in order to meet cellular energy and metabolic demands (133). Their role is even more important in neurons that need a large amount of ATP for the synthesis and secretion of neurotransmitters, to enhance the synaptic plasticity and also to maintain the neuronal membrane potential (131). As a result, impaired mitochondrial function inevitably leads to a pathological state, ranging from subtle alterations in neuronal function to cell death and neurodegeneration. As mentioned before, an age-dependent decrease of brain bioenergetics metabolism together with an impaired redox homeostasis occur during the aging process in individuals with a history of a normal and healthy life (13, 40, 66, 103). In this situation, mitochondrial dysfunction is usually latent until a threshold of perturbations is reached, which eventually results in the disruption of cellular function. On the other hand, this scenario may be accelerated in a context of AD or the threshold of cell impairment in response to abnormal mitochondria may be lower in AD patients (11). In this regard, dysfunctional mitochondria are presumed to compromise neuronal plasticity and neuronal response to metabolic challenges, physiological and environmental cues and the encoding of new memories by lowering the energy charge in neurons (105). In the next subsections, it will be made a survey among the major insidious disturbances of mitochondrial homeostasis in the context of AD (ie, oxidative stress, energy hypometabolism and defects in mitochondrial dynamics, transport and quality control).

Oxidative stress, bioenergetics and energy metabolism in AD

It is recognized that AD has a long latent period before symptoms appear and a diagnosis can be made (227). Further, recent studies demonstrate that the onset of AD is commonly preceded by a transitional state known as mild cognitive impairment (MCI), when there is no significant increase of senile plaques and NFTs (124, 227). As demonstrated, MCI subjects often present increased levels of oxidative stress markers and decreased levels of non-enzymatic antioxidants (22, 94, 170, 180). Likewise, evidence shows that such oxidative stress imbalance directly correlates with severity of cognitive impairment as well as with increasing age (221) and symptomatic progression from MCI to AD (10). In this scenario, oxidative modifications have been proposed as one of the biochemical changes possibly leading to the neuropathology and neuronal dysfunctions and death, generally found in AD (107). As described, ROS-mediated injury, mainly through increased levels of lipid peroxidation markers, namely thiobarbituric acid reactive substances and 4-hydroxynonenal, is found in the brains of AD patients compared to controls (21, 123). Noteworthy, a previous study elegantly demonstrated that mitochondria-derived ROS themselves trigger A β generation by enhancing the amyloidogenic pathway (104). As authors described, this situation will start a vicious cycle of enhanced A β production in the progression of sporadic AD, since A β itself accelerates mitochondrial dysfunction and its own production via

enhanced β -site APP cleaving enzyme 1 (BACE1) activity due to increased ROS levels (104). In fact, as previously proposed, there is an intrinsic correlation between mitochondrial A β levels and mitochondrial dysfunction in different brain regions and also between these parameters and cognitive impairment in AD transgenic mice (51). In this regard, mitochondrial A β levels strongly influence mitochondrial respiratory function, ROS production rates and membrane potential in different brain regions such as frontal cortex and hippocampus (51). Within mitochondria, A β can bind and inhibit A β -binding alcohol dehydrogenase (ABAD) interfering with the respiratory chain function (namely complex IV), altering mitochondrial efficiency in the modulation of cellular properties and increasing ROS production and damage (113). Furthermore, A β was also shown to interact with the mitochondrial protein import machinery and block TOM40 and TIM23 from importing subunits of complex IV into the mitochondria (70, 113), thereby also resulting in affected complex IV activity and in dysfunctional mitochondria.

Using the triple transgenic mouse model of AD (3xTg-AD), investigators were able to demonstrate that, at least in this mice model, mitochondrial dysfunction in frontal cortex is characterized by decreased mitochondrial respiration and by a significant reduction in ATP synthesis in the hippocampus, compared with control (193). Likewise, studies from our laboratory show that brain mitochondrial anomalies present in the 3xTg-AD mice culminate in energy deficits, increased ROS production and susceptibility to mitochondrial permeability transition pore opening (29, 178). Interestingly, data revealed that the 3xTg-AD mice exhibit stronger defects on OXPHOS, synthesis of ATP and ROS production when compared with the age-matched double transgenic littermates (APPxPS2). In particular, authors observed that a decrease in mitochondrial membrane potential is already detected at 8 months of age in triple AD mice compared to 12 months of age in their double AD mice (179). As suggested, such results are indicative that A β and abnormally hyperphosphorylated tau protein may act synergistically to trigger mitochondrial dysfunction in AD (179, 189). Also, others have observed that in 3xTg-AD at 3 months of age, mitochondrial dysfunction occurs prior to the development of amyloid plaque (239). In this work, mitochondrial impairment in brain tissue from 3xTg-AD was characterized by an increase in hydrogen peroxide production and lipid peroxidation as well by a decrease in mitochondrial respiration, and PDH activity, one of the most important enzyme of oxidative metabolism (239).

Energy hypometabolism in association with reduced neuronal expression of several key enzymes of oxidative metabolism in the diseased AD brain are among the best documented abnormalities in AD (227). In fact, low glucose metabolism at baseline and longitudinal glucose metabolism decline are viewed as sensitive measures useful for monitoring change in cognition and functionality in AD and MCI, and are being increasingly adopted to assist diagnosis and used to predict future cognitive decline (11). In this scenario, consistent data demonstrate that several key respiratory mitochondrial enzymes including isocitrate dehydrogenase, PDH, α -KDH and cytochrome oxidase (COX) present a significant reduced expression and/or activity in the AD brain, occurring prior to the onset of memory deficits and the appearance of the two histopathological culprits of the disease (2, 118, 162, 219). As established, the impairment of the respiratory chain, in connection with AD, is mainly due to the decrease in complex IV (COX) activity (19, 28). Such impairment is often correlated with the early manifestation of

A β toxicity and can have as immediate consequences excess oxygen radical production, which damages COX and surrounding structures decreasing ATP synthesis (190, 214). Further, others have demonstrated that energy deficiency causes an increase in the levels of intracellular amyloidogenic APP fragments *in vitro* (59, 62); and leads to an increase in the amyloidogenic APP processing *in vivo* (220). In further support of the importance of metabolic abnormalities in AD pathogenesis, previous studies have demonstrated that using a pharmacological model of energy metabolism inhibition in APP overexpressing transgenic mice (Tg2576), β -secretase-1 (BACE-1) and A β levels become elevated, suggesting that energy deprivation may be amyloidogenic *in vivo* (156). Likewise, *in vitro* glucose deprivation has been shown to induce AD-like changes in hippocampal neurons and in a neuronal cell line by influencing tau phosphorylation (35, 100). Further, starvation-induced hypoglycemia was earlier suggested to be a simple model to study *in vivo* AD-like tau hyperphosphorylation (235). Thus, in line with those observations and, contrary to common concepts, the AD brain “does not follow a suicide but a rescue program” (77). According to this concept, the brain during aging and in the initial stages of AD actively adapts to the progressive energy deprivation through neuronal compensatory responses and downstream adaptations, such as oxidative utilization of ketone bodies, activation of stress-activated protein kinase pathways, A β deposition and tau protein hyperphosphorylation (41, 77, 142). In fact, data show that the most extensive A β deposits and neurons containing NFTs can have an inverse correlation with markers of oxidative stress, despite an obvious history of oxidative damage (153). This suggests that, in early stages of AD, both A β and NFTs may be cellular compensations to the energy crises, a process that eventually culminates in the exhaustion of cell's defense response consequently leading to neuronal degeneration and death (142).

Mitochondrial dynamics in AD

Through the action of mitochondrial “shaping proteins,” mitochondria have the ability to rapidly change morphology and complex networks within the cell in order to better answer the facing demand. In this context, under physiological conditions, mitochondria assume a thread-like or tubular morphology that change to small round organelles through the rapid and reversible coordination of fusion/fission events (16). Being a process extremely organized and balanced in the normal cell, the disruption of this balance results in morphological changes and abnormal function of mitochondria, resulting in cell death (30). In fact, defects in mitochondrial dynamics events, particularly fission, have been identified in a number of human pathologies, including AD (226). In the first study that implicated the involvement of abnormal mitochondrial dynamics in AD, authors observed a pattern of structurally damaged mitochondria, characterized by broken cristae and partial or near complete loss of the internal structure in biopsied AD brain (79). In particular, authors observed a slight but significant increase in mitochondrial size along with a significant decrease in mitochondrial number in these neurons (79). Further studies demonstrated significant changes in the expression of the mitochondrial proteins involved in the fission/fusion processes including dynamin-like protein 1 (DLP1), optic atrophy protein 1 (OPA1), mitofusins (Mfn) 1 and 2 and mitochondrial fission protein 1 (Fis1) in post-mortem AD brains (36, 116, 224). Using the well-known cybrid

cell model, which lacks their own mtDNA and in which exogenous mtDNA from control, AD and MCI patients were introduced, it was found that AD cybrid cells had significant changes in morphology and function, with mitochondria fragmented, misshapen, bleb-like and collapsed away from the mitochondrial network, such changes being associated with altered expression and distribution of Drp1 and Mfn2 (60). Concurrently, others found that cybrids from MCI and AD patients develop bioenergetics and mitochondrial mass adaptations changes and present a mitochondrial fission–fusion balance shifted toward increased fission characterized by increased levels of mitochondrial Drp1 (197). Also, from a plethora of *in vitro* studies it is becoming possible to underscore the dynamic balance of fission and fusion in AD in different experimental models (225). For instance, a recent study in Neuro-2a (N2a) cells overexpressing APP, demonstrated that A β production triggers mitochondrial fragmentation through a decrease in the levels of Mfn1 and Mfn2 without changing the levels of Drp1 (161). In this same context, a previous study revealed that the impaired mitochondrial dynamics in AD seems to result from the colocalization of Drp1 and A β and subsequent increased production of free radicals in AD brains (116). In turn, this elevation of free radicals activates fission proteins Drp1 and Fis1 and causes excessive mitochondrial fragmentation, defective transport of mitochondria to synapses, low synaptic ATP levels and synaptic dysfunction in AD neurons (177). Of note, more recently, Zhang *et al.* (240), using a new technology of 3-dimensional electron microscopy (3D EM) reconstruction to visualize mitochondrial structure in the brain tissue from patients and mouse models of AD, identified a previously unknown mitochondrial fission arrest phenotype that results in elongated interconnected organelles, “mitochondria-on-a-string” (MOAS). Accordingly to this study, instead of the expected excessive mitochondrial fragmentation, AD mouse models presented this MOAS phenotype that increased with disease progression and was more pronounced in animals with multiple FAD mutations (240). Such results prompted authors to suggest that the MOAS phenotype may represent a sustained transition state dynamics allowing for a compensatory adaptation of mitochondria to bioenergetics stress, since it was also observed in animal models and in humans in response to energetics stress associated with AD, hypoxia and aging (240). Taken together, the aforementioned studies highlight the importance of mitochondrial dynamics in AD pathogenesis.

Mitochondrial autophagy and biogenesis in AD

When neurons accumulate dysfunctional mitochondria or face increased metabolic or bioenergetics demands, they must pursue one or a combination of several strategies to ensure sustained function. One response includes mitophagy, a form of autophagy in which defective mitochondria are selectively degraded in double membrane autophagosomes to ensure the maintenance of a healthy mitochondrial pool (184). In brief, autophagic vesicles formed in neurites must be transported back to the cell body by retrograde transport where they fuse with lysosomes. In normal conditions, the autophagic process in neurons is constitutively active and very efficient so autophagosome accumulation is rarely observed (149). In fact, previous studies suggest that in the early stages of AD, the upregulation of autophagy (83) and the increased expression of lysosomal enzymes (33) can act as a protective mechanism to fight cell degeneration (184). However, the presence of A β increases the

number of autophagome-engulfed mitochondria present in neurites and becomes a major intracellular reservoir of toxic peptides in AD brains (39, 150). This is likely due to an increased rate of autophagic sequestration of organelles coupled with a decreased rate of fusion of autophagic vesicles with lysosomes (39). This disrupted autophagosomal and lysosomal function may play an important role in mitochondrial dysfunction and intracellular A β accumulation in AD (190). As firstly described by Hirai *et al.* (79), postmortem AD brain tissue possesses decreased number of mitochondria in vulnerable neurons but increased cytosolic accumulation of mitochondrial markers such as mtDNA and subunit I of COX, suggesting an impaired autophagic lysosomal proteolytic degradation, and a possible leak of sequestered material from the autophagosome vacuoles. Also, elevated levels of mitochondrial components, namely COX and lipoic acid, within autophagosomes were detected in human postmortem brain tissue, suggesting an increase in the rate of mitochondrial degradation by autophagy (140, 141). So, it seems that in AD, the combination of increased autophagy induction and defective clearance of A β -generating autophagic vacuoles creates conditions favorable for A β accumulation in AD (151).

Another major link between mitochondria and neuronal (dys)function concerns mitochondrial biogenesis and disturbances in its major regulator, the peroxisome proliferator-activated receptor gamma (PPAR γ) coactivator 1 α (PGC-1 α), which is the coactivator of nuclear transcription factors (NRFs) 1 and 2 and mitochondrial transcription factor A (TFAM) (202). As pinpointed in literature, brains of patients with neurodegenerative diseases present low levels of PGC-1 α which may underscore mitochondrial dysfunction and oxidative stress (227). Also in AD patients (172) and cell models (195), a downregulation of PGC-1 α and its major target genes has been documented. For instance, data from our laboratory show a decrease in NRF1 and NRF2 levels in 3xTg-AD brain, which may underlie the decrease in ND1 levels, a mitochondrial DNA encoded protein, which is suggestive of a decreased mitochondrial biogenesis (31). Following this line of thought, an increased expression of PGC1 α was considered a compensatory response against A β toxicity in N2a cells treated with A β (24) and in APP/PS1 mice (241). As so, overexpression of TFAM protected SH-SY5Y cells from A β -induced mitochondrial dysfunction (234) whereas PGC-1 α overexpression in N2a neuroblastoma cells promoted a decrease in secreted A β and increased the levels of non-amyloidogenic soluble A β (93). However, in opposite, the cross of Tg19959-AD mice with mice overexpressing human PGC-1 α worsened A β and tau accumulation, led to mitochondrial abnormalities, neuronal cell death and an exacerbation of behavioral hyperactivity in mice (53). As authors discussed, preserving the exquisite balance of PGC-1 α expression and function may be critical to achieve beneficial effects, which may be of relevance when designing therapeutic strategies (53).

Mitochondrial trafficking in AD

Neurons, that are long, excitable and, highly compartmentalized cells, have an intrinsic dependence in the proper distribution of mitochondria to sustain the spatial and temporal demand of energy that differs within the axons and synapses compared to dendrites and the cell body (58, 81, 114). Both synaptic and nonsynaptic mitochondria are synthesized in the neuronal soma and consequently have to be transported to the nerve terminals where the

bioenergetics demands are high in order to fulfill their physiological functions (114). A number of observations, such as a reduced dendritic mitochondrial coverage, a selective and increased degradation of mitochondria by mitophagy, a reduction of synaptic vesicle number at nerve terminals, as well as mitochondria accumulation in the neuronal soma, implicate disturbances in axonal transport in the progression of AD (228). In brief, mitochondrial long-distance transport in axons, to reach all the places that need energy, is driven by kinesin, the primary anterograde mitochondrial motor, and by dynein, primary retrograde mitochondrial motor, powered by ATP hydrolysis that shuttle mitochondria along microtubules (188). As shown in previous studies, an impairment in kinesin-based axonal anterograde transport including the transport of mitochondria is implicated in *in vitro* and in mouse models of AD (102, 167). For instance, a brief treatment of A β monomers and fibrils was demonstrated to be enough to induce significant reduction in motile mitochondria in hippocampal neurons (182); a process likely to occur through the activation of glycogen synthase kinase 3 β (GSK3 β) (182) and consequent increased phosphorylation of kinesin light chains (KLC) (167). Similarly, a study using real-time analysis of vesicle motility demonstrated that isolated axoplasms perfused with soluble intracellular oligomeric A β exhibit inhibition of bidirectional axonal transport as a result of increased phosphorylation of KLC and subsequent release of kinesin from its cargoes (e.g. mitochondria) (166). Others have shown that hippocampal neurons treated with A β -derived diffusible ligands (ADDLs) develop an impaired axonal transport of mitochondria in both anterograde and retrograde directions (226). Likewise, A β overexpression in *Drosophila* decreased mitochondrial velocity in both directions, which is associated with axonal depletion of mitochondria (244). In the same animal model of AD, Folwell *et al.* (57) found that the coexpression of A β with phosphorylated human tau increases tau phosphorylation and exacerbates axonal transport dysfunction. In turn, Du *et al.* (52) found that low levels of A β promote an impairment in anterograde but enhance the retrograde axonal transport of mitochondria, whereas Calkins *et al.* (24) demonstrated a specific impairment in anterograde transport of mitochondria without changes in the retrograde direction in primary neurons from Tg2576 APP transgenic mice. So, from the studies discussed above and others (26, 42, 215), it can be stated that axonal transport defects strongly impact AD-linked pathologic factors. Importantly, studies in 3xTg-AD mice showed that the deficits in axonal transport and axonal swelling precede A β deposition or filamentous tau aggregation, suggesting that such deficits might be early events in AD (136). As the machineries mediating axonal transport of mitochondria and mitochondrial fusion are closely interconnected (138), alterations in the morphology as well as decreased ATP levels *per se* will interfere with axonal trafficking of mitochondria and result in depletion of functional mitochondria in the synapses causing defects in synaptic function and neurodegeneration. Thus, maintaining a healthy mitochondrial network is a major actual goal (91).

MITOCHONDRIAL MEDICINE FOR AD: MITOCHONDRIA AS A THERAPEUTIC TARGET

Albeit the question whether the mitochondrial-related changes contribute to the development of or are a consequence of the disease process remains open, an undoubted link between mitochondrial

dysfunction and AD has been extensively demonstrated. As previously mentioned, in general, mitochondrial perturbation can arise in several ways, e.g. alterations in mitochondrial function, morphology and dynamics associated with A β accumulation and/or ROS production are among the earliest observed pathogenic alterations observed in AD, preceding the formation of amyloid plaques (168). Thus, understanding the mechanisms behind the occurrence of such mitochondrial alterations will likely offer new avenues for preventive or therapeutic strategies. Since the first report of a disease characterized by mitochondrial dysfunction (112) and the emergence of the “mitochondrial medicine” concept (111), medicine has evolved and nowadays it includes pharmacological and lifestyle interventions, both with the rationale of directly or indirectly target mitochondrial dysfunction and its consequences (205).

Pharmacological interventions to target mitochondria in AD

One of the critical goals of pharmacological science is the development of novel and safe drugs for the prevention and treatment of age-related diseases. While AD-related hypotheses postulate several causes and therapeutic targets, no clearly effective disease-modifying interventions are currently recognized (30). With the increasing understanding of the mechanisms underlying mitochondrial dysfunction and its key role in AD pathogenesis, several preventive and therapeutic strategies have emerged.

From antioxidants to TPP⁺ and SS conjugated compounds

Being widely discussed in both the lay press and the scientific literature as health-promoting agents that may protect against various age-related diseases, antioxidants were the first form of mitochondrial medicine intended to treat and/or delay AD progression. In general, antioxidant strategies can be divided into three main categories: 1) free radical scavengers, e.g., vitamins C and E, β -carotene; 2) preventive antioxidants such as metal chelators, glutathione peroxidases and superoxide dismutase (SOD) enzymes; and 3) *de novo* and repair enzymes such as lipases, proteases and DNA repair enzymes (152). Also, nonspecific antioxidants can include melatonin (55), omega-3 polyunsaturated fatty acid (docosahexaenoic acid) (14), curcumin (237), ubiquinone (38) and α -lipoic acid (174). Focused in reducing oxidative stress and/or preventing ROS damage to mitochondria, the use of most of those antioxidants in a preclinical step culminated in very promising results and, for example low-molecular weight antioxidants, such as vitamin E and *N*-acetylcysteine were soon considered as wannabe candidates for clinical trials in AD. However, human clinical experience with such antioxidant neuroprotectants quickly showed some caveats and did not match the expectations (89). For instance, even though numerous cellular and animal models of AD have been developed, one of the major challenges in translating a therapy from a preclinical to a clinical setting is the inability to find an animal model that completely mimics the human nature of the disease (43). Further, another possibility relates with the fact that in the human clinical trials, treatment is often initiated too late in the course of the disease (89). Importantly, as pinpointed elsewhere, when it comes to designing potential antioxidant approaches, cell compartmentalization issues require consideration. Antioxidants

that only access the cytosol may have a limited impact on mitochondrial ROS (203). As so, antioxidant therapy become blunted by the inability to enhance antioxidant levels in mitochondria mainly due to the low permeability of the blood–brain barrier to most of the antioxidants currently used and the difficulty encountered in surpass multiple barriers such as the cell membrane and the outer and inner mitochondrial membranes (135). To circumvent this concern and to better assess whether antioxidant approaches may be valuable therapeutic treatments, researchers have developed strategies to direct antioxidants into mitochondria. One of those strategies is the conjugation of the lipophilic triphenylphosphonium cation (TPP⁺) to antioxidants such as vitamin E, coenzyme Q (MitoQ) and α -lipoic acid. Since these molecules enter into mitochondria several hundred-fold more than natural antioxidants, they will rapidly neutralize free radicals at their source with an improved therapeutic potential (146). In fact, as expected, MitoQ demonstrated effectiveness in attenuating A β -induced neurotoxicity, ROS production and loss of mitochondrial membrane potential ($\Delta\psi_m$) in cortical neurons (134) as well as in preventing the loss of spatial memory and to delay early neuropathological changes in young 3xTg-AD females (134). Likewise, using a *Caenorhabditis elegans* model overexpressing human A β , Ng *et al.* (148) evidenced that the early administration of MitoQ exerts protective effects on lifespan and A β -induced paralysis, observations that were related with the protection of complexes I and IV of the electron transport chain (ETC) (148). Further, MitoQ also revealed to be capable of preventing A β -induced mitochondrial fragmentation, to increase neurite outgrowth in N2a neuroblastoma cells treated with A β , and to enhance synaptic branching and connectivity in primary neurons from AD and wild-type mice (117).

More recently, to overcome the logistics of safe drug delivery to the mitochondria in sufficient amounts and the toxicity associated with high doses, mitochondria-targeted polymeric nanoparticle system (NPs) started to be used (229). Because of its ease of production, flexibility with respect to surface modification and tunable drug release profiles, NPs are becoming an attractive mitochondria-targeting drug delivery system (155). In this context, the conjugation of NPs to TPP⁺, allowed researchers to formulate a targeted curcumin-loaded NPs (127). Previously tested in clinical trials to slow AD progression but without improvements in cognitive decline, curcumin is a well described inhibitor of A β toxicity with antioxidant and anti-inflammatory properties (127). Being mitochondrial-targeted, curcumin-loaded NPs exhibited significantly higher protective effects against A β in human neuroblastoma cells when compared with nontargeted particles or free curcumin (127), revealing the importance of targeting the antioxidant agents to the mitochondria.

Later on, as an alternative to the TPP⁺ strategy, researchers developed the SS peptides, a family of small mitochondria-targeted antioxidant molecules (210). By having a sequence motif that allows them to target mitochondria, even in depolarized mitochondria, these SS peptides display mitochondrial accumulation and ability to scavenge H₂O₂ and inhibit lipid peroxidation (210). SS31, the most extensively studied SS peptide, in particular, is capable of entering mitochondria and of concentrating in the inner mitochondrial membrane, protecting mitochondria against mitochondrial permeability transition pore (mPTP) formation, swelling and cytochrome *c* release (243). Using SS31 in different AD models as N2a neuroblastoma cells treated with A β , primary neurons

from Tg2576 mice and aged Tg2576 mice, a range of effects on mitochondria were observed (25, 117). In particular, SS31 decreased the levels of mitochondrial fission proteins (Drp1, Fis1) and matrix protein, CypD and reduced mitochondrial dysfunction in neurons affected by AD. Further, SS31 enhanced the number of healthy and intact mitochondria, and increased synaptic outgrowth and neuronal branching (117). Meanwhile, SS31 also proved its efficacy by restoring mitochondrial transport and synaptic viability, and decreasing the percentage of defective mitochondria in primary neurons from Tg2576 mice (25), thus indicating that SS31 protects mitochondria and synapses from A β toxicity.

In general, even though the application of these mitochondria-targeted agents to AD is at its early stages and is mainly focused on animal models of AD, the outcomes are positive and worth to follow as potential drugs to enter in clinical trials with AD patients.

Strategies to manipulate mitochondrial quality control and dynamics in AD

Through studies that aim to elucidate the role of mitochondria in AD onset and development it is clear that abnormal mitochondrial dynamics and quality control play an important role in AD neurodysfunction and neurodegeneration. In this scenario, mitochondrial fission inhibitors, such as mdivi-1, ranks itself as a new candidate for AD treatment (60). Using AD cybrids as an *in vitro* AD model, researchers observed that this inhibitor of Drp1 GTPase activity, protected against mitochondrial fragmentation and mitochondrial functional defects, including deficits in complex IV activity, observed in AD cybrid cells (60). Simultaneously, others also reported that mdivi-1 treatment markedly reverses mitochondrial fission and mitochondrial membrane potential loss, cytochrome *c* release and caspase-3 activation caused by A β treatment in BV-2 and primary microglial cells (232).

As the search for novel and potentially effective agents for the treatment of AD, as well as selected promising treatment strategies lasts, researchers strive for new mitochondrial targets. For instance, using an animal model with a genetically manipulated voltage dependent anion channel (VDAC1) protein, Manczak *et al.* (120) showed that a reduction in VDAC levels is associated with a decrease in the activity of Drp1 in neuronal cells and concomitant decrease in mitochondrial fission and prevention of neuronal death, thus suggesting that compounds to target VDAC protein may constitute a novel therapeutic target (119). More recently, others reported that CR6-interacting factor 1 (Crif1), a MIM protein that is key for the translation of mitochondrial OXPHOS subunits and their insertion into the MIM (95), is a key player in A β -induced mitochondrial dysfunction (23). In particular, authors observed that Crif1 is decreased in the brains of AD patients and mouse models and its reduction is associated with massive mitochondrial fission and loss of cristae in an *in vitro* model whereas its overexpression rescues A β -induced disruption of mitochondrial morphology (23). Such promising results prompted authors to propose that Crif1 may serve as a novel therapeutic target in the treatment of AD (23). Using a different strategy, in a recent study, Zhang *et al.* (241) observed that the impairments in mitochondrial homeostasis and the cognitive deficits present in an AD mice model can be reverted by a neural stem cell (NSC) transplantation method. Specifically, NSC transplantation upregulated mitochondrial biogenesis, thus generating more mitochondria with normal function to rescue the

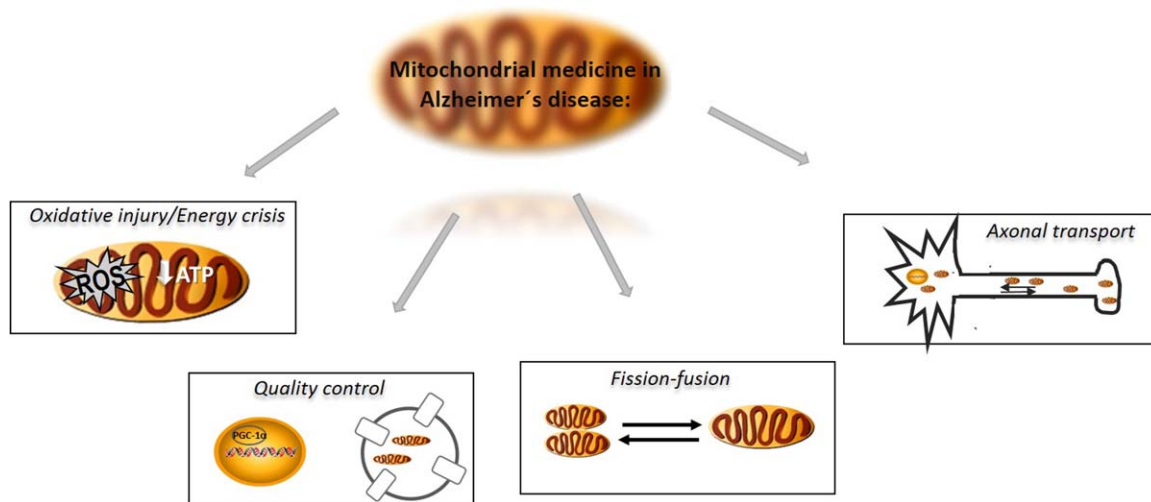


Figure 1. Mitochondrial targets of intervention in Alzheimer's disease. Being actively engaged in a plethora of neurodegenerative diseases, including Alzheimer's disease (AD), the actual compromise of mitochondrial medicine is to find strategies to target and/or manipulate the major insidious disturbances of mitochondrial homeostasis and to directly or indirectly manage their consequences

mitochondrial dysfunction and cognitive deficits found in AD (241). Supported by these and by other similar observations (18, 242), authors point out that a NSC-based therapy targeting mitochondrial biogenesis could be considered a promising therapeutic strategy in AD (241).

Overall, research performed in *in vitro* and animal models of AD highlights several therapeutic targets and pharmacological strategies aimed to reestablish mitochondrial homeostasis (Figure 1). Even though more in-depth studies are needed before translation to human medicine, future studies will hopefully define more clearly which of those strategies are worth to follow.

Lifestyle interventions

It is widely accepted that diet strongly influences the incidence and outcome of major age-related diseases including diabetes, obesity and vascular disease. As posited by a growing body of evidence, brain aging and neurodegeneration are tightly linked with metabolic and energy balance with recent findings extending influences of diet to AD (54, 68). As the main source of energy for the normal function of brain cells, the decline of mitochondria function has been proposed to be a major factor in the loss of brain function with aging and in age-related neurodegenerative disorders (176). As so, strategies to preserve brain mitochondrial integrity and metabolism during aging are often regarded as critical for maintaining healthy brain function, often referred to as healthspan, and for extending lifespan (129). While current therapeutics only temporarily ameliorate the symptoms of AD, but very few affect the underlying disease mechanism (191), a number of epidemiological studies, however, suggest that simple lifestyle changes may be sufficient to slow the onset and progression of AD (169). In this setting, evidence from antioxidant-based trials and pre-clinical studies have shed light to the need of concomitant dietary and lifestyle factors in refining efficacy of antioxidant therapies (37, 164). As so,

in the context of AD in order to reestablish mitochondrial homeostasis. With this goal, mitochondrial medicine point to reset the mitochondrial interconnected features of quality control (autophagy and mitochondrial biogenesis), mitochondrial axonal transport, fission/fusion processes, bioenergetics (energy production) and reactive oxygen species production.

non-pharmacological interventions, such as calorie restriction, and/or physical activity have been gaining recognition as a very effective means to extend lifespan and delay the appearance of age-related pathological conditions, notably those associated with brain functional decline (5).

Calorie restriction and calorie restriction mimetics in AD

"Eat less, age well, and remember well," the mantra that is becoming principal in cutting-edge research on aging and human health, highlights for the importance of dietary nutrition intervention in human aging and age-related diseases, including AD (106, 110). Defined as a moderate reduction in calorie intake of 20%–40% in the absence of malnutrition, calorie restriction (CR) or dietary restriction (DR) is nowadays the only nongenetical experimental manipulation that is known to preserve metabolism in aging process and extend the lifespan of a broad range of species, spanning from yeast to rodents and non-human primates (5, 84, 200). By activating a class of enzymes known as sirtuins, CR posits itself as having remarkable neuroprotective properties (173). For instance, in AD mouse models, CR has been found to diminish AD symptoms (130, 145, 163) whereas in a primate model, CR increased neurotrophic factors and attenuated behavioral deficits (128). In a close way, others show that CR ameliorates neurodegenerative phenotypes assessed by object recognition and contextual fear conditioning tests and reduces tau hyperphosphorylation in cDKO (conditional double knockout) AD mice (231), suggesting a potential therapeutic value of CR for patients with AD. In this context, even though an epidemiological study by Luchsinger *et al.* indicates that individuals with a low-calorie intake may have a reduced risk of developing AD (110), the utility of CR as a strategy for humans is somehow hampered by the degree and length of restriction required and the will of patients to go through a reduction in

food ratio (9). Thus, it become of great interest to understand the mechanisms by which CR modifies organismal physiology, particularly in light of current efforts to develop CR mimetic (CRM) compounds (34, 85, 198). By definition, CRM are alternative chemical compounds that can “mimic” the biological effects of CR without significantly reducing calorie intake. And so, the ideal CRM would be an agent consumed in food or water that would delay death and age-associated diseases without requiring a change in calorie intake (84). Of note, even though these compounds could also be included in the pharmacological subsection of this manuscript, their effects will be discussed here in the context of its CR-related effects. Due to its ability to mimic the metabolic, hormonal and physiological effects of CR, activate stress response pathways and reduce the incidence of age-related diseases, CRM are often associated with an improvement in the overall health- and lifespan of the organism (200).

One of the most popular compounds with CR properties is resveratrol (15). With a previous epidemiological link to longer life in humans (109, 160), resveratrol by activating sirtuins was found to decrease aging-dependent cognitive decline and pathology in AD animal models (92, 122). Further, this CR mimetic was also able to protect cells against A β -induced ROS production and DNA damage *in vitro* (85, 187). Also, in a rat model of sporadic AD, resveratrol was found to prevent cognitive impairment induced by an intracerebroventricular injection of streptozotocin (194). More recently, a randomized, placebo controlled, double-blind, multicenter 52-week phase 2 trial of resveratrol in individuals with mild to moderate AD revealed that resveratrol consumption is safe, well-tolerated and alters some AD biomarker trajectories (216; clinicaltrials.gov ID NCT01504854).

Nevertheless, even having a well-documented neuroprotective role in several models of neurodegenerative diseases (200), the exact mechanism by which CR and its mimetics influences aging and/or AD remains unclear. Still, one of such mechanisms that is currently accepted relates with mitochondria and mitochondria function, namely with the prevention of oxidative damage and mitochondrial ROS production, improvement of metabolic parameters, increased mitochondrial biogenesis through activation of the SIRT1-PGC1 α pathway and resistance to cellular stress (47, 121). As demonstrated, by activating SIRT1, resveratrol reduces defects in electron transfer in ETC, decreases mitochondrial ROS, increases oxygen consumption and maintains ATP production in neurons (8, 9). As nicely described by López-Lluch *et al.* (108), mitochondria under CR conditions show less oxygen consumption, reduced membrane potential and generate less ROS than controls, but remarkably they are able to maintain their critical ATP production. A process that, accordingly to the authors, critically relies in the activation of diverse regulatory pathways greatly enhances stress resistance via the SIRT1 pathway and markedly improves bioenergetics through the activation of the PGC-1 α pathway (108). Being responsible for the transcription of cellular programs regulating mitochondrial respiration, oxidative stress defense and adaptive thermogenesis, PGC-1 α is considered a master regulator of mitochondrial biogenesis and metabolism. So, it can be suggested that CR and/or CRM through its direct effects in PGC1 α promote an adaptive response in the cell that accounts for an increase in the number and size of mitochondria as well as an enhancement in the respiratory rate. Overall, those changes will elevate the oxidative buffer capacity of the cell, augmenting its resistance to conditions

of stress (121). As mentioned before, mitochondrial dysfunction, especially a defect in mitochondrial biogenesis, is an early and prominent feature of AD (172), so it seems that compounds and/or interventions that reverse the PGC1 α deficit, or otherwise enhance PGC1 α activity or expression, constitutes a feasible AD therapeutic target (6).

Considering that energy sensing pathways appear critical in regulating aging in a number of model systems, in a general way, CRM are targeted to energy pathways that mimic the physiological responses of CR and to enhance stress responses (84). As so, besides resveratrol, to date, several candidate CRM have been proposed and studied to treat and/or delay AD, among others are included, 2-deoxy-D-glucose (2-DG), sirtuin activators and the inhibitors of the mammalian target of rapamycin (mTOR) (212). Due to the structural similarity between 2-DG and glucose, 2-DG is transported by glucose transporters into the cell where it binds to, but since it cannot be phosphorylated by hexokinase, it will induces a compensatory rise in alternative substrates, primarily ketone bodies by the liver and, activates an alternative energetic pathway in brain (238). As previously demonstrated, in a similar way to CR, the 2-DG treatment was shown to mediate neuroprotection in models of AD (67). For instance, Yao *et al.* (238) using 3xTgAD mice fed with 2-DG, observed that this dietary intervention was responsible for an improvement in brain mitochondrial bioenergetics paralleled with a reduction in oxidative stress. Also, dietary 2-DG promoted a reduction in A β generation and increased mechanisms of A β clearance, further suggesting dietary 2-DG as a disease-modifying intervention to delay progression of bioenergetics deficits in brain and associated amyloid burden (238).

One key sensor of nutrient availability in higher organisms is mTOR. With previous evidence that CR inhibits mTOR signaling in multiple species including mice (90, 230), mTOR has become a candidate mediator of at least some of CR's beneficial effects (137). In this set, strategies for the inhibition of mTOR as a therapeutic strategy in AD have been tested in animal models of the disease (99, 236). For instance, some studies offer strong evidence that rapamycin, the well-known inhibitor of mTOR, and its derivatives can decrease amyloid burden in APP-overexpressing mouse models, when administration is performed in early stages of the disease (115) and ameliorate tau pathology in the 3xTg-AD and P301S tau transgenic mice models (157). Further, other compounds with structural similarities to resveratrol, RSVA314 and RSVA405, were found to inhibit mTOR activity, and to promote the degradation of A β by the autophagic-lysosomal machinery (223).

Physical exercise in AD

Besides the widely accepted beneficial effects of exercise in enhancing a range of physical indices from balance, bone density, strength and endurance to lipid profiles, blood pressure and cardiovascular health (87, 213), it is now recognized that regular exercise holds important benefits for both affective experience and cognitive performance regardless of age (80). In fact, physical inactivity and a sedentary lifestyle are nowadays considered significant risk factors to develop dementia and neurodegeneration (101, 175). In this scenario, over the past years, retrospective and prospective epidemiological studies documented brain health benefits of exercise on the development of AD and dementia of any type (159). In concordance with those studies, data obtained from several animal

models of AD show that physical exercise is a simple behavioral intervention sufficient to inhibit the development of AD-like neuropathology and to improve cognitive behavior (1, 37, 61, 218). Of note, at least in AD mouse models, the age of the mouse and thus level of pathology must be considered when selecting an appropriate exercise regime, with maximal effects on AD pathology observed when exercise is initiated prior to the appearance of A β plaques or at an early-mild stage of plaque deposition (183).

Albeit the significant outcomes regarding cognitive function reported in both epidemiological and animal studies, the cellular and molecular mechanisms underlying this exercise-induced protective phenotype in the brain are still elusive (125). For instance, even though the brain is a non-contractile tissue, neuronal function seems to be indirectly influenced by an increase in energy metabolism (50). In this setting, brain mitochondrial metabolism seems to be central in the cross-tolerance phenomena by which physical exercise confers neuroprotection (125). As reported, exercise induces important brain mitochondrial adaptations in order to sustain increased metabolic demands (48). Among those, are included an increased content and/or activity of several enzymes involved in aerobic energy production (48, 49, 96), increased activity of mitochondrial complexes I, III and IV (147), decreased expression/activation of several pro-apoptotic proteins (217), increased mitochondrial biogenesis (201) and antioxidant capacity (27), as well as, alterations in proteins involved in mitochondrial dynamics, apoptosis and autophagic signaling (126). As so, previous data confirm that endurance training attenuates neuronal cell apoptosis involved in the pathogenesis of AD by promoting reductions in brain cytochrome c, Bax, caspase 3 and 9 levels, and an elevation of heat shock protein 70 (HSP70) (37, 218). Further, as suggested by others, other important mitochondrial bioenergetics adaptations associated with voluntary exercise concerns an increase in the mitochondrial uncoupling protein 2 (UCP2) gene expression (48). Previously reported as exerting an important protection against A β toxicity and oxidative stress (88), UCPs, mainly UCP2, are often regarded as an effective strategy in the regulation of mitochondrial biogenesis by decreasing ROS overproduction, increasing ATP generation and improving calcium homeostasis (7).

From the aforementioned studies, one can hypothesize that strategies aimed to modulate mitochondrial function and trigger neuroprotective mechanisms represent effective strategies against brain aging and several age-related neurodegenerative diseases (Figure 1). However, further investigation is required to elucidate the real impact of these strategies in AD patients (7, 48, 125).

CONCLUSIONS

As outlined in the studies discussed above, it is clear that mitochondria are at the heart of the neurodegenerative process occurring in AD. Studies reveal that mitochondria play a major role in several stages of AD progression; they can be considered triggers as well as targets of the neurodegenerative cascade that characterizes AD. The ongoing research in this field continues to shed light on the mechanisms involved in the maintenance of mitochondrial integrity and their relevance for disease, making them interesting and valuable targets for therapeutic interventions. Until now, due to the intricate mechanisms involved in disease pathogenesis most of the clinical trials carried out with the investigational drugs available

terminated without being successful and so, there is an urge to develop strategies aimed to delay the onset or to slow down AD progression. Due to the deep involvement of mitochondria in AD, a great expectation is being posed in mitochondrial medicine to directly manage the major insidious disturbances of mitochondrial homeostasis occurring in AD. Nevertheless, and considering AD a multifactorial pathology, a mono-target approach like those currently used is insufficient to foster positive results, and so it must be stressed out that clinical trials should try to combine efforts to include multiple intervention arms, such as co-administration of mitochondrial-directed antioxidants with other promising therapeutic options (e.g. lifestyle interventions). Even though more in-depth studies are needed before pharmaceutical industry can apply this knowledge to human medicine, the continuous advances in AD research field facilitate and accelerate the development of more effective preventive or therapeutic strategies to fight this devastating disease.

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