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Review

# Mitochondria and Mitochondrial Cascades in Alzheimer's Disease

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**Abstract**. Decades of research indicate mitochondria from Alzheimer's disease (AD) patients differ from those of non-AD individuals. Initial studies revealed structural differences, and subsequent studies showed functional deficits. Observations of structure and function changes prompted investigators to consider the consequences, significance, and causes of AD-related mitochondrial dysfunction. Currently, extensive research argues mitochondria may mediate, drive, or contribute to a variety of AD pathologies. The perceived significance of these mitochondrial changes continues to grow, and many currently believe AD mitochondrial dysfunction represents a reasonable therapeutic target. Debate continues over the origin of AD mitochondrial changes. Some argue amyloid-β (Aβ) induces AD mitochondrial dysfunction, a view that does not challenge the amyloid cascade hypothesis and that may in fact help explain that hypothesis. Alternatively, data indicate mitochondrial dysfunction exists independent of Aβ, potentially lies upstream of Aβ deposition, and suggest a primary mitochondrial cascade hypothesis that assumes mitochondrial pathology hierarchically supersedes Aβ pathology. Mitochondria, therefore, appear at least to mediate or possibly even initiate pathologic molecular cascades in AD. This review considers studies and data that inform this area of AD research.

Keywords: Alzheimer's disease, bioenergetics, cascade, cytochrome oxidase, mitochondria

## INTRODUCTION

Over 40 years ago, electron microscopy (EM) pictures of Alzheimer's disease (AD) brains revealed altered mitochondrial infrastructures [1, 2]. Initial reports, though, offered limited speculation into the cause or significance of this basic observation. Later studies confirmed and extended the finding [3, 4]. In the early 1980 s, fluorodeoxyglucose positron emission tomography (FDG PET) studies showed brains from AD patients utilized less glucose than brains from control subjects [5–8], which piqued interest in a potential metabolism component for this disease [9–12]. Investigators subsequently attempted to explain reduced AD brain glucose utilization. Proposed hypotheses included impaired blood-brain barrier glucose transport [13], reduced tissue energy requirements due to reduced synaptic activity, a tissue-loss artifact, and lesions of energy metabolism enzymes [10].

Whether related or not to reduced FDG PET glucose utilization, around this time and shortly thereafter, studies in fact did reveal activity deficiencies in several bioenergetic flux-related enzymes. Some studies noted reductions in glycolysis enzymes [14]. Initially implicated mitochondria-localized enzymes included pyruvate dehydrogenase complex (PDHC) and α-ketoglutarate dehydrogenase complex (KGDHC) [15, 16]. Functional differences from control subjects were later demonstrated in additional Krebs cycle enzymes [17].

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A single study from 1987 analyzed oxygen consumption in brain biopsy homogenates from dementia subjects [18]. Interestingly, under submaximal conditions oxygen consumption from the dementia patient homogenates exceeded that of the control subject homogenates. Mitochondrial uncoupling in the dementia subject homogenates potentially contributed to this perhaps unexpected finding. In contrast, PET, when used to quantify brain oxygen consumption *in vivo*, showed decreased oxygen consumption by AD brains [19, 20]. Consistent with the oxygen consumption PET data, Parker et al. in 1990 reported reductions in AD subject cytochrome oxidase (COX) activity [21].

Still, the origin and consequences of these seminal but descriptive observations remained unclear. An obvious consideration was whether mitochondrial or other bioenergetic lesions contributed to AD, or whether they simply represented a byproduct of the disease. Based on assumptions made by the amyloid cascade hypothesis, first proposed in the early 1990 s [22, 23], the latter possibility seemed more likely. Even when viewed from this perspective, though, accumulating data from the past two decades suggest if indeed mitochondrial-bioenergetic dysfunction represents a byproduct of more fundamental AD events, this dysfunction may yet play an important and possibly relatively upstream role in mediating AD dysfunction and degeneration. Other data, though, also argue mitochondrial dysfunction could represent an independent or perhaps even primary event in AD.

## EVIDENCE OF SECONDARY MITOCHONDRIAL CASCADES IN AD

Sequential cleavages of the amyloid-β protein precursor (AβPP) produce a peptide byproduct called Aβ. A popular line of AD research argues Aβ, and perhaps specifically oligomeric conformations of a 42 amino acid-long Aβ species, initiates neurodegeneration [24, 25]. Cultured cells maintained in the presence of Aβ show reduced electron transport chain enzyme activities [26]. Aβ also impairs respiratory chain function in isolated mitochondria [27–29]. While such data indicate potential important relationships between Aβ and mitochondria might exist, they do not compellingly address the AD-relevance of an Aβ-induced mitochondrial lesion, let alone the presence of a resulting mitochondrial cascade.

A report from Cardoso et al. more directly assessed this question [30]. The authors added Aβ to the medium of human neuronal NT2 cells, which induced cell death. They similarly added Aβ to the medium of NT2 ρ0 cells, NT2 cells previously depleted of their mitochondrial DNA (mtDNA). Because they lack mtDNA, ρ0 cells do not produce key respiratory chain subunits and are respiration-incompetent. The Aβ treatment did not harm the ρ0 cells. These studies suggested mitochondria in general, and the respiratory chain specifically, may mediate Aβ toxicity. This study was perhaps the first to make a case for a possible Aβ-induced mitochondrial cascade in AD.

Subsequently, studies revealed AβPP and Aβ colocalize with mitochondria [31–37]. Prominent early observations came from models that featured an artificial expression of AβPP, and of Aβ overproduction. Because artificial expression or overproduction of a protein may affect its intracellular targeting, it is reasonable to question the physiologic relevance of these findings. However, analyses show mitochondria from AD subject autopsy brains do appear to contain Aβ [33, 35, 38, 39].

Research by Yan and colleagues sought to define how Aβ located within mitochondria might mediate mitochondrial dysfunction and result in a downstream pathologic cascade. In the first of these studies, that of Lustbader et al., Aβ bound a dehydrogenase protein the authors called the Aβ-binding alcohol dehydrogenase (ABAD) [31]. Blocking ABAD-Aβ physical interactions mitigated oxidative stress and apoptosis. In the second study, that of Du et al., Aβ bound cyclophilin D (cypD), a component of the mitochondrial transition pore. CypD knock-out reduced oxidative stress and apoptosis, and cypD knock-out preserved cognitive performance in AβPP transgenic mice [36]. The results of these studies are consistent with the possible presence of functionally important Aβ-induced, mitochondria-mediated pathologic cascades in AD.

AβPP itself contains a reported mitochondrial targeting motif [40–44]. When AβPP accesses mitochondria, it partly passes through the mitochondrial protein import apparatus, including the translocase of the outer mitochondrial membrane 40 kilodalton (TOMM40) protein pore. This passage ultimately does not complete, due to the presence of an AβPP acidic domain, and the mitochondrial import-arrested AβPP both clogs the import infrastructure and protrudes from the mitochondria and into the cytoplasm. The presence of arrested AβPP in the mitochondria

(with its associated C-terminal end extended into the cytosol) reportedly reduces COX activity [40].

Altered calcium homeostasis represents yet another fundamental physiologic alteration Aβ can induce [45, 46]. In both cell and animal experimental models, Aβ appears to both enhance the ability of calcium to access the cytoplasm, and reduce the cell's ability to lower its cytosolic calcium levels. Elevated calcium interferes with mitochondrial function, which reduces ATP production. An inhibition of oxidative phosphorylation additionally depolarizes mitochondria, which further impairs the ability of cells to buffer calcium loads. Based on this, some propose an AD "calcium hypothesis [47]." Under this scenario, Aβ-induced changes in calcium homeostasis adversely impact mitochondrial function, and through this drive other AD-associated changes in brain function. Disrupted calcium handling, therefore, could represent a mechanism via which secondary mitochondrial cascades initiated by Aβ ultimately drive this disease.

Other studies performed using transgenic mice designed to model human AD infer a meaningful role for mitochondrial damage (at least in these models). For example, Reddy et al. found that in mice expressing a mutant human AβPP transgene, an apparent compensatory upregulation of respiratory chain enzyme subunits preceded both behavioral change and Aβ plaque deposition [48].

Recent reports indicate either AβPP overexpression or Aβ exposure affects various aspects of mitochondrial function [43]. In both AβPP transgenic mice and cultured cells treated with Aβ, the balance between mitochondrial fission and fusion shifts in favor of fission [49–52]. Fewer mitochondria also distribute throughout dendrites. Analyses of brains from AD patients show similar changes [3]. If Aβ causes these mitochondrial perturbations in autopsy brains, and these mitochondrial perturbations in turn contribute to neuron dysfunction or degeneration, they could represent evidence of an Aβ-induced, mitochondria-mediated cascade [43, 53]. Figure 1 illustrates potential causes and consequences of a secondary mitochondrial cascade.

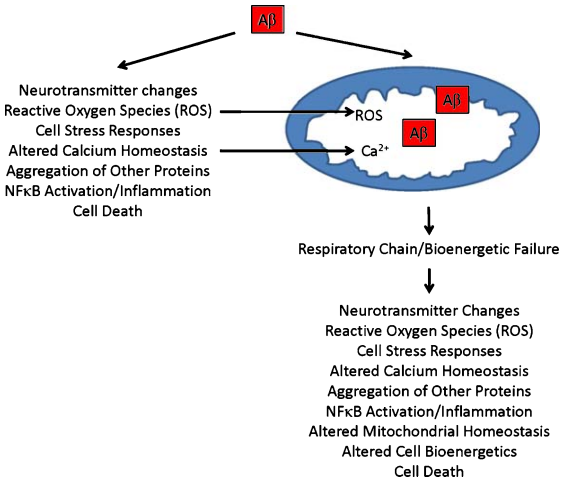


Fig. 1. Secondary mitochondrial cascade. Secondary mitochondrial cascades are compatible with the amyloid cascade hypothesis, and could mediate Aβ toxicity. As illustrated, Aβ can directly introduce various AD-associated functional changes and pathologies, and directly or indirectly cause mitochondrial dysfunction. Aβ-induced mitochondrial dysfunction, in turn, could further contribute to or initiate additional AD-associated functional changes and pathologies.

## EVIDENCE OF A PRIMARY MITOCHONDRIAL CASCADE IN AD

Interestingly, some of the more influential discoveries of altered energy metabolism enzymes arose from studies of non-brain tissues, and were later subsequently documented in brain parenchyma. Parker and colleagues first documented lower AD COX activity in platelets [21]. Blass, Gibson, and colleagues first showed reduced KGDHC activity in fibroblasts, and lowered transketolase activity in red blood cells [16]. Reports from the mid-1980 s noted altered patterns of glucose and oxygen consumption in fibroblasts from AD subjects [54, 55].

While biochemical defects in AD brain could represent a consequence of neurodegeneration, neurodegeneration *per se* should not directly cause specific biochemical defects outside the neuro-axis and in non-degenerating tissues. Other structural brain changes that could affect energy metabolism, such as synaptic loss, similarly should not directly cause non-neural biochemical defects. AβPP or Aβ could perhaps drive biochemical changes in different tissues provided their expression and production occurs in those tissues. To this point, AβPP does appear outside of the brain, but expresses as different isoforms. Aβ also exists in multiple tissues outside of the brain, including blood vessels, skin, subcutaneous tissues, intestine, and muscle [56]. To what extent peripheral processing of AβPP to Aβ occurs locally, or whether Aβ simply exports to these tissues, remains unknown although it increasingly appears that some tissues do locally generate Aβ. Two examples include muscle and platelets [57–59].

The presence of distinct, definable biochemical features outside the brains of AD subjects and within multiple tissues suggests at a biochemical level, AD is not a brain-limited process or event. At the very least, it is hard to see how a single, stochastic Aβ oligomerization-aggregation event occurring within the brain could alter either AβPP homeostasis or energy metabolism across multiple tissues. Even if altered Aβ homeostasis does contribute to non-brain bioenergetic or mitochondrial features seen in AD subjects, the question of why Aβ homeostasis itself changes across multiple tissues warrants consideration.

An alternative possibility is that altered energy metabolism in AD subjects exists independently of AβPP or Aβ, and that altered energy metabolism drives changes in AβPP and Aβ homeostasis. Existing data support this possibility. In cell culture experiments, Gabuzda et al. found COX inhibition shifts AβPP processing toward the amyloidogenic pathway that ultimately produces Aβ [60]. Mitochondria-generated reactive oxygen species may play a particularly important role in shifting AβPP processing to Aβ [61]. Other cell culture studies suggest interfering with cell bioenergetics shifts AβPP processing away from its non-amyloidogenic pathway, and presumably towards the amyloidogenic pathway [62, 63].

One study found transgenic mice that concurrently expressed a mutant human AβPP gene and proof reading-defective mtDNA polymerase γ (mtPOLG) showed enhanced Aβ plaque deposition [64]. Due to the presence of the mutated mtPOLG transgene, these mice acquire excess somatic mtDNA mutations and mitochondrial dysfunction [65, 66]. This finding links mitochondrial dysfunction to plaque deposition. Other studies performed using AD mouse models report similar links, although the direction of the relationship differed. Moraes and colleagues previously reported, in two studies using different approaches, that interfering with respiratory chain assembly actually reduces Aβ plaque deposition [67, 68]. In one case, the authors induced mitochondrial dysfunction through targeted knockout of the gene for COX10, which encodes a farnesyltransferase required for COX assembly. In the other, they expressed a mitochondria-targeted restriction enzyme that cleaves mtDNA. It is unclear why driving mitochondrial dysfunction through different approaches produced either increased or decreased plaque deposition. Most likely, methodologic factors are relevant. In the Moraes group studies, the mice produced less respiratory chain infrastructure, and with mtPOLG driving mitochondrial dysfunction aberrant rather than less respiratory chain infrastructure likely resulted. Other potential factors could include differential effects on cell unfolded protein responses. Regardless, studies such as these link mitochondria to plaque deposition.

In another study, Scheffler et al. used a selective breeding strategy to create AβPP transgenic mice that varied primarily in the origin of their mitochondria [69]. More specifically, the authors created mice that contained mitochondria from different mouse strains. Ultimately, the various groups of mice differed only in their mtDNA sequences. This study reflected two previous studies performed in cytoplasmic hybrid (cybrid) cell lines, in which the investigators generated human cell lines containing mtDNA from different individuals [70, 71]. Both studies created a group of cybrid lines in which the mtDNA came from individuals with AD, and a group of cybrid lines in which the mtDNA came from age-matched, control subjects. In each case, the cybrid lines that contained the mtDNA form the AD subjects produced (or at least retained) more Aβ.

The use of cybrid cell lines that contain mtDNA from AD subjects, herein referred to as "AD cybrids," warrants additional consideration. Cybrid studies in general are consistent with the presence of a primary mitochondrial cascade in AD. AD cybrid cell lines were first created in the 1990 s to address the specific question of whether mtDNA contributes to lower platelet COX activity in AD subjects [72, 73]. First, the investigators removed the endogenous mtDNA from human neuronal cell lines, either the SH-SY5Y neuroblastoma cell line or the NT2 teratocarcinoma cell line, thereby creating SH-SY5Y and NT2 ρ0 cell lines [73–75]. Next, they isolated platelets from blood samples taken from subjects with and without AD. Briefly maintaining platelets from individual subjects with ρ0 cells in the presence of a detergent allows for the mixing of platelet and ρ0 cell cytoplasms. Selecting specifically for cells that contain a ρ0 cell nucleus and platelet mitochondria results in the isolation of a unique cybrid line. Because the nuclear component of cybrid cell lines prepared using a particular ρ0 cell line is similar if not identical, different cybrid cell lines generally contain the same nuclear DNA [76]. As for components transferred from platelets, the only component that can perpetuate over time is the mtDNA that is contained within the platelet mitochondria. As the cells divide, all other platelet-derived components degrade and dilute. Ultimately,

the most fundamental difference between different cybrid cell lines prepared on the same nuclear background is their mtDNA sequence. The transfer of platelet mitochondria, and the mtDNA they contain, to ρ0 cells to create cybrid cells restores the ability of the former ρ0 cells to respire. In this case, the respiratory chains of the cybrid cells jointly consists of subunits encoded from the nuclear DNA of the original cell line nucleus and the mtDNA from the particular platelet donor [76]. Between cybrid cell lines prepared on the same nuclear background, the nuclear DNA-encoded respiratory chain subunits are presumably identical. The mtDNA-encoded respiratory chain subunits, though, differ because mtDNA sequences vary between individuals. Differences between mtDNA sequences affect respiratory chain function, so that cybrid cell lines containing mtDNA from different donors vary in terms of respiratory function. This in turn leads to differences in mitochondrial function, which mediates effects on a variety of cell characteristics.

Studies generally report that although COX activities between individual cybrid cell lines prepared from AD or control subject platelets do overlap, mean COX activity in groups of AD cybrids is lower than that of the comparative control groups [72, 73, 77–79]. In these experiments, a transfer of AβPP or Aβ is much less likely to drive the observed lower mean COX activity. Rather, cybrid data indicate mtDNA at least partly accounts for the observed reduction in AD subject platelet mitochondria COX activity. The magnitude of the difference between COX activity means varies between studies, but generally reflects a 15–40% activity reduction [80]. Methodologic differences likely contribute to this range.

Importantly, differences between AD and control cybrid lines are not limited to COX activity. While values among AD and control lines generally overlap for measured parameters, mean values for a given group of AD cybrid lines occasionally are higher or lower than their comparative control group cybrid lines [70–73, 77–79, 81–97]. Increased parameters include markers of oxidative stress, Aβ production, stress pathway activation, NFκB activation, apoptotic signaling, and mitochondrial fission. Decreased parameters include mitochondrial membrane potential, ATP levels, oxygen consumption, glycolysis flux, calcium homeostasis, and peroxisome proliferator-activated receptor γ related complex α (PGC1α). These differences tend to recapitulate features observed in studies of brains from

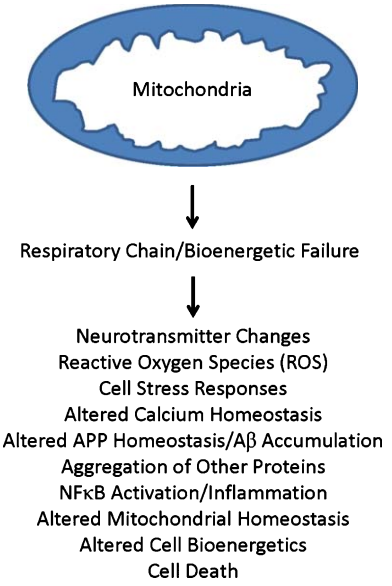


Fig. 2. Primary mitochondrial cascade. A primary mitochondrial cascade is incompatible with the amyloid cascade hypothesis. Under this scenario, impaired mitochondrial function and associated bioenergetic changes alter Aβ homeostasis and lead to an accumulation of Aβ. Aβ may or may not in turn contribute to the development of other AD-associated functional changes and pathologies.

AD subjects. Because the only consistent difference between cybrid lines of AD and control groups is whether the mtDNA they contain originated from AD versus control subjects, and the mtDNA within the cybrid lines derived from a non-brain, non-degenerating tissue, and it is not clear how transferred AβPP or Aβ could account for the observed biochemical and molecular changes, cybrid data support the presence of a primary mitochondrial cascade in AD (Fig. 2).

The "mitochondrial cascade hypothesis" represents one formal attempt to explain how mitochondria may serve as the primary generator of AD [98–101]. This mitochondrial cascade hypothesis incorporates data from genetic, biochemical, molecular, cell biology, animal, clinical endophenotype, and epidemiologic studies. Also, as mitochondrial function generally declines during aging [102], it tries to bridge AD and aging research by providing a platform that potentially explains why advancing age represents the single greatest AD risk factor [103]. It is important to note various versions of this general postulate exist, and that some of these versions similarly identify a role for mtDNA, be it in the form of acquired, somatic mtDNA mutations or in the form of

inherited variants [21, 104, 105]. The mitochondrial cascade hypothesis, with the intent of acknowledging data that support both views, proposes inherited mtDNA variants influence mitochondrial function and aging, and in the brain somatic mtDNA mutations that influence mitochondrial function accumulate with advancing age.

## ASSUMPTIONS AND LIMITATIONS OF THE (PRIMARY) MITOCHONDRIAL CASCADE HYPOTHESIS

A primary mitochondrial cascade hypothesis neither depends on nor addresses the question of whether Aβ, in any of its forms, contributes to AD dysfunction or degeneration. Rather, it infers mitochondrial function or cell bioenergetic states meaningfully alter either AβPP production, intracellular AβPP targeting, processing of AβPP to Aβ monomers, the formation of Aβ oligomers or fibrils, or the removal of Aβ. Toxic or non-toxic amyloid cascades may exist, but if so, they are secondary events.

Similarly, a primary mitochondrial cascade hypothesis neither depends on nor addresses the question of whether aggregation of tau protein into tangles, or the presence of tau tangles themselves, contributes to AD dysfunction or degeneration. Instead, it infers mitochondrial function or cell bioenergetic states meaningfully alter either tau production, intracellular targeting, processing, post-translational modification, oligomer or fibril formation, or removal. Tangles or other tau protein derivatives may or may not function in a toxic fashion, but if they do, it represents a secondary event.

Undoubtedly, in the neurodegenerative disease field protein aggregation and mitochondrial dysfunction commonly occur. Existing literature does not resolve whether one of these pathologies consistently drives the other. It is nevertheless worth mentioning new reports that indicate on a fundamental level, bioenergetic states and mitochondria profoundly influence protein aggregation. In one study, Patel et al. showed that ATP functions as a "hydrotrope" [106, 107]. Essentially, its hydrophobic nucleotide portion associates with hydrophobic protein segments, while its hydrophilic phosphate group maintains the complex in a soluble state. At physiologic ATP concentrations, its hydrotrope properties prevent proteins that tend to self-aggregate from self-aggregating. As ATP concentrations fall to intermediate levels, this hydrotrope effect weakens and oligomers form. As ATP levels fall to low levels, fibrils then begin to assemble. In another study, Ruan et al. showed that mitochondria act as a sink for aggregation-prone proteins, and mitochondrial proteases degrade those proteins following their importation [108]. A loss of this "mitochondria as guardian in cytosol" (MAGIC; a term used by the authors to describe this phenomenon) function could in general promote protein aggregation.

When it comes to MAGIC, though, it is also conceivable that a primary protein overload may drive mitochondrial dysfunction, and through this instigate a secondary mitochondrial cascade. This could prove especially pertinent to cases of AD that occur in the presence of deterministic AβPP, presenilin 1 (PSEN1), or presenilin 2 (PSEN2) gene mutations. It is therefore possible that some of what we now consider AD arises through a primary mitochondrial cascade, while some of what we now consider AD essentially involves a secondary mitochondrial cascade.

It is still possible that mitochondrial cascades (either as primary or secondary events) are in fact not important in AD. If so, it would imply that observed differences between mitochondria from AD and non-AD brains constitute a disease-associated but non-contributing biomarker of the responsible process, or an end-stage artifact of the responsible process. It is more difficult to account for non-brain differences in mitochondria and mitochondrial function, but potential explanations are still possible. For example, a genetic parameter that influences AD risk could lead to a physiologically diffuse yet unrelated change in mitochondria or their function. APOE or perhaps TOMM40 genotypes could to some extent conceivably mediate such a phenomenon [109–112]. Similarly, over the course of many years a lifestyle characteristic could lead to a diffuse yet unrelated change to mitochondria.

Despite these caveats, extensive literature does reveal mitochondria from AD subjects (i.e., the cybrid literature) or inducing mitochondrial dysfunction recapitulates a number of AD-associated molecular events. Mitochondria also undoubtedly critically contribute to an array of cell processes, including oxidative stress, calcium homeostasis, and cell death. These factors would seem to argue anatomically widespread changes to mitochondrial function in AD subjects represent more than simply coincidental events or disease artifacts.

## IMPLICATIONS FOR THE TEMPORAL AND SPATIAL POSITIONING OF AD BIOMARKERS

Investigators currently use specific cerebrospinal fluid (CSF) and neuroimaging-derived biomarkers to document transitions within the brain, and to link those transitions to AD. These include measurements of CSF Aβ and tau protein, PET-based visualization of fibrillar Aβ and tau, and magnetic resonance imaging-inferred reductions in hippocampal volume. It is possible to temporally order these biomarker changes [113, 114]. Initial shifts occur in the Aβ measurements, which manifest as decreased CSF Aβ (particularly $A\beta_{42}$) or as an arbitrarily defined, suprathreshold accumulation of parenchymal Aβ plaques. Next, CSF tau levels fall, and parenchymal, fibrillar tau accumulations start to extend beyond the medial temporal lobes. After this, hippocampal volumes shrink and shortly thereafter performance on cognitive tests typically falls below expectations.

This temporal ordering is consistent with the idea of a primary amyloid cascade, but relevant studies also contribute data that are harder to incorporate. For example, Aβ plaque deposition typically precedes cognitive decline by 1–3 decades [113–118]. This suggests Aβ-induced physiologic damage lags substantially behind its physical deposition, and infers Aβ toxicity is at most subtle. In response to this, some now speculate Aβ itself is insufficient to cause the disease, but instead initiates AD by triggering a critical change in tau biology [114, 119, 120]. This change in tau biology, either by itself or by working in conjunction with Aβ (more specifically oligomers of $A\beta_{42}$), ultimately destroys the hippocampus and causes cognitive decline. On a conceptual level, this possibility would not rule out the presence of a secondary mitochondrial cascade.

This temporal scheme emphasizes biomarker changes investigators can detect over the course of a longitudinal study, or by analyzing different cross-sectional studies. Some studies, though, suggest when it comes to predicting or at least monitoring AD risk, particular AD-relevant biomarkers potentially predate low Aβ CSF levels or Aβ plaque deposits [121]. In one relevant study, investigators analyzed diary entries from young nuns (written mostly at the beginning of their third decade) for idea density and grammatical complexity [122]. Nuns who wrote with lower idea density were more likely to develop AD in old age. In another study, individuals who reached their highest level of employment in their third or fourth decade were subsequently more likely to develop AD than individuals who continued to advance through employment hierarchies [123]. The authors speculated in this case, a relative premature peaking in one's employment trajectory could possibly reflect a manifestation of incipient AD. Additional biomarker studies report similar findings and further suggest mitochondrial function or energy metabolism could constitute a relevant factor. Middle-aged individuals with an increased lifetime AD risk, as defined by the presence of an APOE4 allele or a maternal family history (a marker of mtDNA inheritance) of the disease, are more likely to demonstrate AD-like changes on FDG PET scans [124–126]. As a group, middle-aged people with AD-affected mothers also have reduced (relative to middle-aged individuals without an AD-affected mother) platelet mitochondria COX activity and exhibit brain regions with reduced volumes [127–132]. Middle-aged children of AD mothers who also carry an APOE4 allele perform relatively less well on memory testing [133]. Other similarly structured studies using different biomarker measurements show essentially consistent findings [134–138]. These data suggest when it comes to brain aging and AD, inherited or acquired energy metabolism parameters could predate Aβ changes. Results from at least one direct biochemical study of brain tissue are consistent with these findings from living subjects [139]. On a conceptual level, this is compatible with the presence of a primary mitochondrial cascade (Fig. 3).

Biomarker spatial distributions also warrant consideration. Within the brain, Aβ plaque and tau tangle deposition follow predictable anatomical patterns. Initially, plaques occur predominantly within the brain's default mode network, a region that features high levels of aerobic glycolysis [140]. A secondary mitochondrial cascade would infer that Aβ causes aerobic glycolysis in this region, while a primary mitochondrial cascade would infer aerobic glycolysis in some way enhances Aβ deposition. Similarly, a secondary mitochondrial cascade would infer that outside the brain, brain-derived Aβ causes changes to mitochondrial function [80] and calcium homeostasis [141–143], while a primary mitochondrial cascade would infer those changes arise independent of Aβ, and perhaps drive local Aβ deposition [70, 72].

Ultimately, the cascades that drive AD biomarkers will influence how we define this disease. If a primary amyloid cascade drives AD, then persons who lack an AD clinical phenotype but have amyloid

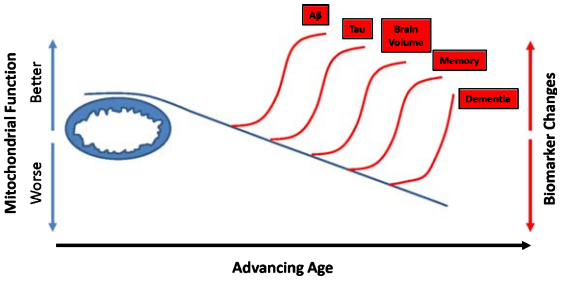


Fig. 3. Could mitochondrial function influence recognized AD biomarker changes? Studies that track dynamic biomarker shifts suggest changes in Aβ (lower CSF levels or plaque accumulation) precede tau changes (lower CSF tau or tangle accumulation beyond the medial temporal regions). Reductions in brain volume (hippocampal volumes) follow Aβ and tau changes. Declining memory abilities and dementia eventually occur. Additional data, though, suggest metabolism-relevant characteristics may distinguish high AD risk individual from low AD risk individuals before detectable Aβ changes occur. Mitochondrial function also changes with advancing age. It is reasonable to consider whether mitochondria define thresholds at which these biomarker changes begin to manifest.

plaques may arguably have AD; a current construct refers to this state as "preclinical AD" [144]. Further, those with AD phenotypes that lack amyloid plaques and an obvious alternative clinical diagnosis arguably do not have AD; a current construct refers to this state as "suspected non-Alzheimer pathology" (SNAP) [145]. Conversely, if a primary mitochondrial cascade drives AD, then the presence of plaques would essentially function as a biomarker (rather than a cause) of changing brain bioenergetics. Those with a primary energy metabolism failure, a typical AD phenotype, but not plaques could thus potentially represent "plaque-negative" AD cases.

## IMPLICATIONS FOR THERAPEUTIC DEVELOPMENT

Mitochondrial and amyloid cascades could coexist. A secondary mitochondrial cascade may mediate damage caused by a primary amyloid cascade, or amyloid produced as part of a primary mitochondrial cascade could itself cause harm. Therapies targeting Aβ may ultimately reveal the extent to which Aβ contributes to AD. If these approaches robustly benefit AD patients, then Aβ likely plays a substantial and potentially proximal role. If Aβ toxicity plays a minimal or downstream role, such approaches will probably confer at best minimal benefits. Clinical efficacy would not rule out, though, the concomitant presence of a mitochondrial cascade. Obviously, if a primary amyloid cascade drives AD by inducing a secondary mitochondrial cascade, then eliminating Aβ would in general help to prevent the secondary mitochondrial cascade. AD investigators who believe in a secondary mitochondrial cascade are meanwhile also developing interventions that would ideally interrupt very specific parts of an Aβ-dependent mitochondrial cascade. Some efforts include deploying small molecule inhibitors of previously documented Aβ-mitochondria interactions [37, 146]. Other interventions currently in development intend to block Aβ-induced changes in mitochondrial physiology. Preventing an Aβ-induced increase in mitochondrial fission represents one such approach [147].

Addressing a primary mitochondrial cascade may require unique strategies. These strategies could focus on preventing age-related declines in mitochondrial function, for example through exercise or diet. Pharmacologic manipulations that enhance aerobic or other aspects of mitochondrial function, or overall cell bioenergetics, could prove beneficial [148]. Proposed interventions include the use of molecules that can enhance bioenergetic fluxes or increase mitochondrial mass [149].

## CONCLUSIONS

AD features mitochondrial and bioenergetic alterations that could contribute to the development or progression of the disease. Upstream pathologies, including Aβ, may influence mitochondrial and bioenergetic function, and thereby initiate a secondary mitochondrial cascade. Alternatively, a primary mitochondrial cascade might represent a proximal cause of many AD cases, and directly perturb vital brain functions or introduce other pathologies that secondarily disrupt normal brain physiology and cause neurodegeneration.

Data support the possibility of both primary and secondary mitochondrial cascades in AD. The presence of a secondary mitochondrial cascade does not contradict, and is consistent with, the potential presence of a primary amyloid cascade. The presence of mitochondrial changes outside of the brain in AD, the fact that Aβ plaques deposit in brain regions defined by specific bioenergetic conditions, that mitochondrial and bioenergetic changes seem to temporally precede detectable changes in Aβ homeostasis, and the ability of mitochondrial dysfunction to affect a variety of AD-associated pathologies argue in favor

of a primary mitochondrial cascade. Regardless of which view is correct, both primary and secondary mitochondrial cascades currently represent reasonable AD therapeutic targets.

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