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APOE4 Impairs Oxidative Phosphorylation and Cholesterol Metabolism in Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is strongly associated with mitochondrial dysfunction, especially in the presence of the *APOE4* allele. Here I review recent research on the connection between APOE4 and impairment of mitochondrial oxidative phosphorylation (OXPHOS). In *APOE4* carriers, decreased basal respiration rates suggest reduced mitochondrial respiration, and studies have reported downregulation of mitochondrial proteins critical for OXPHOS function. Furthermore, cytotoxic APOE4 fragments in neurons inhibit OXPHOS by decreasing mitochondrial membrane potential. In addition, APOE4-induced cholesterol accumulation in astrocytes and oligodendrocytes correlates with mitochondrial OXPHOS impairment. Lastly, I highlight the potential of statins, which are known to lower cholesterol levels, as a therapeutic treatment. Given the crucial role of mitochondrial dysfunction in AD pathology, a better understanding of how APOE4 affects mitochondrial OXPHOS would aid in the development of new strategies to slow down AD progression.

Introduction

Alzheimer's disease (AD) is a major neurodegenerative disease, accounting for 60–80% of all dementia cases. There are two main types of AD: autosomal-dominant AD (ADAD) and late-onset AD (LOAD) (Barber, 2012; DeTure & Dickson, 2019). The former is primarily caused by mutations in genes encoding amyloid precursor protein (APP), presenilin 1 (PSEN 1), presenilin 2 (PSEN 2), and others (Barber, 2012; DeTure & Dickson, 2019). ADAD is relatively rare, representing less than 1% of all AD cases. In contrast, LOAD is much more common, with the ε4 allele of the apolipoprotein E (*APOE*) gene being the strongest risk factor for developing this form of AD (Barber, 2012; DeTure & Dickson, 2019).

The *APOE* gene expresses glycoproteins composed of 299 amino acids that primarily function in lipid transport and metabolism (Mahley, 1988). The *APOE* gene has three alleles, namely the ε2 (*APOE2*), ε3 (*APOE3*), and ε4 allele (*APOE4*), which differ at two specific sites from each other. *APOE2* is associated with a decreased risk of developing AD, *APOE3* is considered neutral, while *APOE4* is strongly linked to an increased risk. In fact, *APOE4* is estimated to be present in over 50% of all AD cases, although allele frequencies vary amongst ethnic groups (Farrer et al., 1997).

AD pathology progresses slowly over an individual's lifetime and involves highly complicated mechanisms. The disease is believed to begin decades before clinical symptoms manifest. In the central nervous system, AD pathology is characterized by the accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles, along with increased neuroinflammation, abnormal glial activity, and synaptic loss (DeTure & Dickson, 2019). APOE4 has been shown to exacerbate many of these abnormalities in the brain, either through a loss of essential functions or a gain of toxicity (Blumenfeld et al., 2024; DeTure & Dickson, 2019).

Research has also highlighted the role of mitochondrial dysfunction in the development of AD pathology (Wang et al., 2020). Accumulating evidence suggests that APOE4 hinders mitochondrial function through the downregulation of mitochondrial respiratory complex proteins, the presence of toxic APOE4 fragments, and cholesterol

buildup. This paper will discuss recent findings that collectively support the connection between APOE4 and mitochondrial dysfunction as well as a possible treatment option involving statins.

APOE4 Affects Cellular Respiration

Overview of Cellular Respiration

Cellular respiration is a crucial metabolic process that converts glucose into ATP. It occurs in three stages, namely glycolysis, the citric acid (Krebs) cycle, and the electron transport chain (ETC) (Mahley, 2023; Osellame et al., 2012). First, glycolysis occurs in the cytoplasm, where glucose is broken down into pyruvate, NAD^+, and ATP. These products are transported into the mitochondrial matrix where the Krebs cycle generates additional ATP and produces new molecules such as NADH, FADH2, and CO_2. Lastly, the ETC occurs in the inner mitochondrial membrane, where electrons pass through proteins through a series of redox reactions and release energy. This energy creates a proton gradient, which is used to generate ATP through a process known as chemiosmosis (Cooper, 2000). Together, the ETC and chemiosmosis constitute oxidative phosphorylation (OXPHOS) in mitochondria. Impaired mitochondrial function can disrupt both the Krebs cycle and OXPHOS, leading to deficiencies in glucose metabolism and ATP production.

Lowered Oxygen Utilization in *APOE4* Carriers

OXPHOS involves a series of redox reactions that consume oxygen. One study investigated cytoplasmic hybrid cell lines where endogenous mitochondrial DNA was removed from SH-SY5Y cells and replaced with platelet mitochondria from human subjects with and without AD (Silva et al., 2013). The AD cell lines showed decreased basal oxygen consumption, suggesting reduced respiratory flux (Silva et al., 2013). Additionally, an oxygen consumption rate assay on human induced pluripotent stem cell (iPSC) derived astrocytes revealed decreased basal respiration levels in *APOE4*-expressing astrocytes compared to *APOE*3-expressing astrocytes (Lee et al., 2023), further suggesting lower OXPHOS activity due to reduced oxygen utilization.

APOE4 Downregulates Proteins Involved In OXPHOS

OXPHOS is composed of five complexes (complex I through complex V), each composed of several protein subunits. Transportation of these protein subunits to the inner mitochondrial membrane and assembly into their respective complexes is a delicate process critical for OXPHOS function (Vercellino & Sazanov, 2022). For example, cytochrome oxidase (COX) is responsible for initiating the formation of complex IV (Kish et al., 1992). Notably, COX activity is reduced in the frontal, temporal, and parietal lobes in the brains of AD patients (Kish et al., 1992).

A recent study using Neuro-2a cells, which are mouse neuroblasts used to model human neuropathology, investigated the abundance of these protein subunits (Orr et al., 2019). Proteomics analysis revealed that 33 out of 65 detected subunits were significantly downregulated in *APOE4*-expressing cells compared to *APOE3*-expressing cells (Orr et al., 2019). Additionally, none of the subunits showed increased expression (Orr et al., 2019). Together, these findings suggest that APOE4 contributes to decreased OXPHOS through reduced levels of complex I–V protein subunits.

APOE4 Fragments in Neurons Affect Mitochondrial Membrane Potential

While APOE is mainly expressed by astrocytes, it is also expressed in neurons. One study crossbred loxP-floxed *APOE* knock-in (*APOE*-fKI) mice with Synapsin 1-Cre (Syn-1-Cre) mice to enable conditional deletion of the *APOE*

gene. Afterwards, deletion of the *APOE* gene specifically in neurons led to a 20% reduction in total APOE protein levels in the cortex of both *APOE3*-fKI/Syn-1-Cre and *APOE4*-fKI/Syn-1-Cre mice (Knoferle et al., 2014). This also prevented spatial learning deficits and memory impairment in *APOE4*-fKI/Syn-1-Cre mice, bringing their performance in line with that of *APOE3*-fKI/Syn-1-Cre mice (Knoferle et al., 2014). These results suggest that neuronal APOE4 has detrimental effects on brain functions despite low abundance.

Studies have shown that within neurons, APOE proteins undergo cleavage by an unidentified protease (Chang et al., 2005; Nakamura et al., 2009). This process creates APOE4 fragments that inhibit mitochondrial function. For instance, one type of APOE4 fragment (amino acids 1–272) binds to UQCRC2, cytochrome C1, and COX4I1 (which are all subunits of mitochondrial complexes) and inhibits the activities of complex III and complex IV (Nakamura et al., 2009). *In vitro* experiments have shown that eliminating either of the receptor-binding region (135–150) or the lipid-binding region (241–272) of APOE4 abolishes neurotoxicity (Chang et al., 2005), indicating both regions are necessary for toxic effects on neurons. Additionally, it was found that the lipid-binding region of APOE4 mediates interaction between fragments and mitochondria, suggesting that the cytotoxicity of APOE4 fragments is partially owing to mitochondrial dysfunction (Chang et al., 2005).

When staining Neuro-2a cells with MitoTracker Deep Red 633, a fluorescent dye that highlights active mitochondria, fluorescence intensity was decreased by 25% in cells expressing APOE4 fragments compared to those expressing full-length APOE4 (Chang et al., 2005). Since only mitochondria with normal membrane potential can effectively store MitoTracker, this suggests that mitochondria in APOE4 fragment-expressing cells have reduced membrane potential. Importantly, mitochondrial membrane potential is vital to OXPHOS, as it helps establish the transmembrane potential of hydrogen ions required for ATP production (Zorova et al., 2018).

Discovery of APOE4 fragments has enhanced our understanding of the remarkable toxicity associated with neuronal APOE, but several questions remain that require further research. Identifying the protease responsible for APOE cleavage could be critical for developing effective protease inhibitors. Studies also show that *APOE3* mice have less fragment buildup (Nakamura et al., 2009), suggesting that APOE4 structure may play a key role in promoting cleavage. Moreover, the relationship between APOE4 fragments and mitochondrial membrane potential is not yet fully understood. Further research into these issues could help to elucidate the harmful effects of APOE4 fragments on mitochondria.

Impact of APOE4 On Glycolysis

In addition to reduced OXPHOS levels, TaqMan gene expression profiling of cortical tissue from 6-month-old female hApoE-TR mice revealed that the enzymes hexokinase 1 (Hk1) and hexokinase 2 (Hk2) are significantly downregulated in *APOE4*-carrying mice relative to *APOE3*-carrying mice (Wu et al., 2018). Hexokinase plays a key role in glucose metabolism by catalyzing glucose phosphorylation. As a result, basal glycolysis rate is significantly reduced in *APOE4*-carrying mice compared to *APOE2*- and *APOE3*-carrying mice (Wu et al., 2018). The negative effects on glycolysis appear to be exacerbated by an imbalance in NAD^+ and NADH levels; *in vitro* experiments demonstrate that the ratio between NAD^+ and NADH is altered in *APOE4*-expressing cells, with NAD^+ levels decreased by 25–30% and NADH levels increased by 10–15% (Orr et al., 2019).

Interestingly, other studies report increased rates of glycolysis in *APOE4* carriers. One study using cultures of human iPSC-derived astrocytes observed that glycolysis levels, measured by extracellular acidification rate, were elevated in *APOE4* astrocytes compared to *APOE3* astrocytes upon glucose treatment (Lee et al., 2023). Another study found increased glycolysis levels in the brains of female *APOE4*-KI mice compared to female *APOE3*-KI mice (Farmer et al., 2021). These findings suggest that the effects of APOE4 on glycolysis may depend on cell types (astrocytes versus neurons) and experimental conditions (*in vitro* versus *in vivo*).

Accumulating evidence shows that cerebral glucose hypometabolism occurs early in AD pathology (Wu et al., 2018). Since this abnormality can be observed decades before clinical symptoms emerge (Wu et al., 2018), this suggests that APOE4 may initiate a cascade of changes affecting both glycolysis and OXPHOS from very early on.

Over time, factors such as aging, stress, and injury alter protein expression levels, potentially resulting in fluctuating periods of increased and decreased glycolysis.

Cholesterol Accumulation Leads to Mitochondrial Dysfunction

Cholesterol Accumulation in Lysosomes of Astrocytes

One of the primary functions of APOE is to regulate lipid transport. Due to a lower affinity for lipid binding and transport in APOE4, increased cholesterol buildup is observed in *APOE4* carriers relative to *APOE3* and *APOE2* carriers. Notably, *in vitro* studies show that APOE2 is the most effective at promoting cholesterol efflux in neurons, followed by APOE3, with APOE4 being the least effective (Michikawa et al., 2000).

Cholesterol accumulation affects various organelles like lysosomes. In fact, a recent study using cultures of human iPSC-derived astrocytes observed that cholesterol accumulation primarily occurs in the lysosomes of astrocytes (Lee et al., 2023). After a 4-day application of methyl b-cyclodextrin (MβCD), an agent known to reduce lysosomal cholesterol, lysosomal function was restored (Lee et al., 2023). After a 14-day application of MβCD, levels of OXPHOS returned to normal, while rates of glycolysis remained higher than normal (Lee et al., 2023). MβCD treatment also led to the removal of all mitochondrial structural abnormalities observed, such as increased mitochondrial area and circularity, as well as a restoration of proteins involved in mitophagy (Lee et al., 2023). This study suggests a strong connection between cholesterol buildup and various aspects of mitochondria like structure, mitophagy, and OXPHOS.

Cholesterol Accumulation in The Endoplasmic Reticulum of Oligodendrocytes

A recent study revealed abnormal cholesterol accumulation in APOE4-expressing oligodendrocytes, which are cells responsible for axon myelination and metabolic support (Blanchard et al., 2022). Using oligodendroglia-iNeuron co-cultures, *APOE4/4* co-cultures showed reduced myelin-based protein (MBP) staining near neurofilaments compared to *APOE3/3* co-cultures (Blanchard et al., 2022). Since MBP is vital for the generation of myelin sheaths, this suggests that APOE4 inhibits axon myelination. Application of cyclodextrin, a molecule known to reduce intracellular cholesterol accumulation in Niemann-Pick disease type C (Ottinger et al., 2014), resulted in a reduction of BODIPY-cholesterol staining in *APOE4/4* oligodendrocytes (Blanchard et al., 2022). Additionally, cyclodextrin treatment significantly increased MBP localization near neurofilaments (Blanchard et al., 2022), suggesting that reducing cholesterol accumulation can improve myelination.

In iPSC-derived *APOE4/4* oligodendroglia without treatment, approximately 2% of BODIPY-cholesterol staining was found near endosomes, 18% near lysosomes, and 80% near the endoplasmic reticulum (ER) (Blanchard et al., 2022). This contrasts with the finding that cholesterol accumulation predominantly occurs in the lysosomes of astrocytes (Lee et al., 2023). These results suggest that the location of cholesterol buildup depends on cell type and motivates further research into the impact of cholesterol accumulation in other cell types like neurons.

Notably, the ER interacts with mitochondria through the mitochondrial-associated membrane (MAM) complex, an organelle network where mitochondria are tethered to the ER by specific proteins (Mahley, 2023). The MAM is vital to cellular bioenergetics (Schreiner & Ankarcrona, 2017), indicating that MAM dysfunction may disrupt OXPHOS. *APOE4* carriers exhibit abnormal levels of the proteins involved in the tethering process (Mahley, 2023), potentially leading to changes in mitochondria-ER interaction. Consequently, cholesterol accumulation in the ER of oligodendrocytes due to APOE4 could impair mitochondrial function through MAM dysfunction.

Treatment With Statins to Reduce Cholesterol and AD Pathology

Statins are drugs that hinder HMG-CoA reductase, the enzyme responsible for the rate-limiting step of cholesterol synthesis (Feingold, 2000). This inhibition leads to a decrease in cholesterol and an upregulation of low-density lipoprotein (LDL) receptors (Feingold, 2000). These LDL receptors bind to LDL molecules to facilitate their disintegration by lysosomes, resulting in lower LDL levels and less cholesterol (Feingold, 2000).

Clinical studies have previously provided differing results regarding the effect of statins to treat AD pathology, indicating that statin therapy produces heterogeneous results. In one study, some participants experienced a reversal of benefits of statin therapy upon discontinuation and cognitive-specific adverse drug reactions that also subsided after stopping treatment (Evans & Golomb, 2009). However, other patients experienced a reversal in their dementia or AD diagnosis, suggesting individualized benefits of statin treatment (Evans & Golomb, 2009). Another study assessed statin effects on a variety of neurodegenerative diseases, including AD, dementia, and multiple sclerosis (Torrandell-Haro et al., 2020). In this study, the percentage of people that developed AD without statin treatment was 2.37%, while the percentage of people that developed AD with statin treatment was 1.10% (Torrandell-Haro et al., 2020).

A recent clinical study observed statin therapy with a different approach; 4,807 participants were categorized based on whether they carried the ε4 allele and whether they initiated statin therapy (Rajan et al., 2024). The cumulative incidence of AD followed similar trajectories across all groups except for the "*APOE* ε4 allele with no statin use" group, which had a higher cumulative incidence of AD (Rajan et al., 2024). This data suggests that statin initiation has minimal effects on AD pathology in non-*APOE4* carriers and largely reverses the harmful effects of carrying the *APOE4* allele. One possible explanation stems from increased cholesterol buildup in *APOE4* carriers compared to *APOE2* and *APOE3* carriers. However, it remains unclear why statin treatment appears to have no significant effect on AD pathology in non-*APOE4* carriers.

This recent study (Rajan et al., 2024) may also offer explanations for the results of previous studies that did not consider *APOE* as a factor when delivering statin treatment. As it is estimated that more than half of AD dementia cases involve *APOE4* (Farrer et al., 1997), this may explain the change in the percentage of people developing AD from 2.37% without statin treatment to 1.10% with statin treatment (Torrandell-Haro et al., 2020). Overall, further research is needed to investigate whether the effects of statin are truly dependent on *APOE* gene type. Such research could reconcile previous clinical findings and strengthen the connection between *APOE* alleles and cholesterol accumulation effects.

Conclusion

Strong evidence suggests a connection between mitochondria dysfunction and the development of AD pathology. Studies indicate that reduced mitochondrial OXPHOS is an important part of AD etiology, driven by APOE4-induced downregulation of specific proteins involved in cellular respiration. Additionally, the generation of APOE4 fragments in neurons decreases mitochondrial membrane potential, which hinders OXPHOS and contributes to neuronal APOE4 toxicity. Research on cholesterol accumulation in astrocytes and oligodendrocytes further suggests that cholesterol buildup causes mitochondrial deficits. Importantly, statin treatment, which reduces cholesterol level, may restore OXPHOS function and alter the progression of AD pathology in *APOE4* carriers to resemble that of *APOE3* carriers.

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