Retinal pigment epithelium lipid metabolic demands and therapeutic restoration

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Abstract:

One of the defining features of the retina is the tight metabolic coupling between cells such as photoreceptors and the retinal pigment epithelium (RPE). This necessitates the compartmentalization and proper substrate availability required for specialized processes such as photo-transduction. Glucose metabolism is preferential in many human cell types for adenosine triphosphate generation, yet fatty acid β-oxidation generates essential fuel for RPE. Here, we provide a brief overview of metabolic demands in both the healthy and dystrophic RPE with an emphasis on fatty acid oxidation. We outline therapies aimed at renormalizing this metabolism and explore future avenues for therapeutic intervention.

Keywords:

Beta-oxidation, fatty acids, gene therapy, metabolic coupling, metabolism, mitochondria, retina, retinal pigment epithelium

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Introduction

Retinal pigment epithelium metabolism

In the young healthy retina, photoreceptors and the retinal pigment epithelium (RPE) are highly metabolically coupled. Both of these cell types are specialized to support their individual functions while also providing the nutrients and substrate to allow their counterpart to function properly. The photoreceptors typically function in a highly glycolytic manner, consuming large quantities of glucose that is supplied by the neighboring choroid, and in turn produce lactate. Photoreceptors undergo a process known as outer segment disk shedding to release cell components photodamaged in light to prevent their toxic accumulation, losing around 10% of their outer segment mass daily.[1] The RPE uses lactate, which furthers oxidative phosphorylation (OXPHOS) and shed outer segments as its fuel source. Lactate has been shown to suppress glucose consumption in the RPE which spares more glucose for glycolysis in photoreceptors.[2,3] This lactate consumption is linked to the prevention of long-term oxidative damage,[4] to which the RPE is particularly prone given its proximity to the choroid and preferential metabolic pathways.

To break down the light-damaged shed outer segment pieces, the RPE contains specialized phagocytes which recycle the components back into usable fuel sources.[5] Each RPE cell is in direct contact with ~25–30 photoreceptors and is responsible for the high rate of phagocytosis to ensure nutrient cycling, which allows the proper retinal metabolic demands to be met.[6,7] Kwon and Freeman illustrate some of the more complex metabolic coupling phenomena that occur between the photoreceptors and RPE.[8] Once the outer segment disks are phagocytosed, they must be processed further before their recycling back to the photoreceptors. Early

experiments with cultured porcine RPE indicated that the major pathway through which this occurs is β-oxidation.[9] This process is quite similar to the one in hepatocytes of the liver, in which fatty acids are broken down into β-hydroxybutyrate, a ketone body. Work done by Lattin *et al.* revealed that RPE cells express an enzyme, hydroxymethylglutaryl-coenzyme A (CoA) synthase 2, which is essential for the production of these ketone bodies, strongly supporting prior notions of this metabolic pathway.[10] Subsequent research showed that the ketone bodies produced in the RPE through β-oxidation pathways are preferentially released apically, which supports the notion that these bodies are recycled back to the photoreceptors for downstream oxidative metabolism.[11] These findings validate the theory of fatty acid metabolism being tightly coupled between the photoreceptors and RPE [Figure 1]. The resultant products from mitochondrial β-oxidation fuel the TCA cycle and provide adenosine triphosphate (ATP) which supports RPE cell energetic demands. Additional findings from Adijanto *et al.* point toward monocarboxylate transporters as the main carrier of ketone bodies.[11] This phenomenon of fatty acid and ketone metabolic coupling in various cell types has also been seen between astrocytes and neurons.[12]

The healthy RPE is also rich in peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) which Iacovelli *et al.* showed highly upregulates genes associated with fatty acid metabolism.[13] PGC-1α is a master regulator of mitochondrial activity as it heavily drives OXPHOS and fatty acid β-oxidation. PGC-1α is also partially responsible for mitigating some of the oxidative stress in the RPE through upregulating various antioxidant enzymes as described by Iacovelli *et al.*[13] While two similar pathways
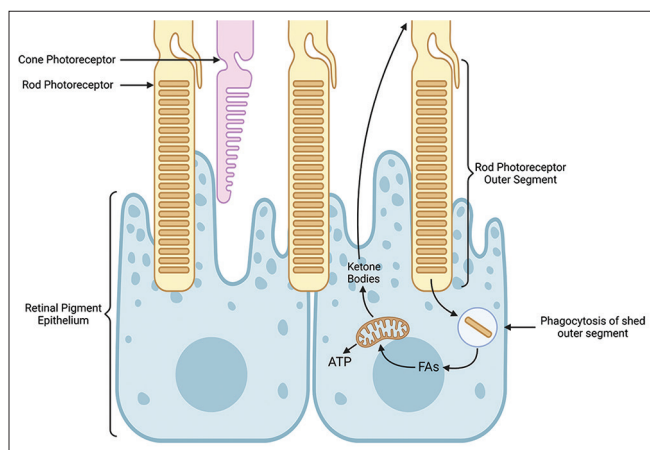
for fatty acid β-oxidation exist[14] (peroxisome-mediated and mitochondria-mediated), evidence suggests that there is a preference for the mitochondrial pathway in the RPE as the peroxisomal pathway fails to yield ATP.[15] While energy production favors mitochondrial fatty acid oxidation, both peroxisomal and mitochondrial oxidative pathways are required for proper visual function.[16,17] For proper mitochondrial metabolism of fatty acids and downstream ketone body production, fatty acids are transported inside mitochondria by carnitine palmitoyltransferase 1 (CPT-1). These transport and fatty acid oxidative pathways can be suppressed as a response to nutrient availability by malonyl-CoA.[18,19] Other evidence supports the concept that prolyl hydroxylase domain proteins play a role in activating downstream metabolic targets to convert acetyl-CoA into malonyl-CoA, which acts as a precursor for fat synthesis and directly inhibits mitochondrial fatty acid uptake by CPT-1.[19]

Loss of Lipid Metabolism in Retinal Degeneration and Aging

The retina is an extension of the central nervous system, and it was originally suggested that glucose is the main fuel source as seen in the brain.[20] However, findings from Cohen and Noell suggested that the majority of energy production in the retina was generated from alternative metabolic pathways.[21] In 2016, research showed that retinal fatty acid β-oxidation accounted for a large proportion of energy production despite a lower efficiency when compared to glucose metabolism.[22] Since fatty acid β-oxidation plays a central role in energy production, it is likely that disruptions to this metabolic pathway impair visual function. Indeed, disorders in this pathway are highly linked to retinopathy[23-25] however disorders associated with glucose transport and uptake show no signs of visual deterioration.[26] One of the key factors associated with proper fatty acid uptake and metabolism is very low-density lipoprotein receptor (VLDLR). Healthy RPE cells express high levels of VLDLR naturally but mutations in this receptor result in mitochondrial dysfunction and downstream progressive retinopathy.[16,23,24,27] Other intermediate work has shown that VLDLR deficient mice have a significant reduction in fatty acid metabolites.[22] As mentioned, the role of peroxisomal fatty acid oxidation is intriguing, as it yields no ATP and therefore should be less preferential to mitochondrial oxidative pathways. Interestingly, disruptions to peroxisomal β-oxidation prevent the oxidation of longer chain fatty acids.[28] These findings suggest that one role of peroxisomes in the RPE is to shorten longer chain fatty acids to enable mitochondrial oxidation.[16] Slightly more upstream of direct inability to metabolize fatty acids, the improper clearance and subsequent accumulation of the outer segment pieces in the RPE have been shown to form lipofuscin that, over

Figure 1: Overview of fatty acid metabolism in the retinal pigment epithelium. Light-damaged shed outer segment disks are phagocytized in the retinal pigment epithelium and broken down to fatty acids. Retinal pigment epithelium mitochondria use fatty acids in a process known as β-oxidation to generate adenosine triphosphate and ketone bodies which are shuttled back to photoreceptors to further glycolytic intermediates and subsequent energy production therein (created on Biorender.com)

long periods of time leads to various retinal disorders.[29] Disorders in RPE fatty acid oxidation impair a major source of energy production and often result in primary RPE retinopathy followed by secondary photoreceptor death.

When fatty acid β-oxidation pathways are disrupted, driven at times by mitochondrial dysfunction, it can lead to downstream retinopathies including retinitis pigmentosa (RP), age-related macular degeneration (AMD), and diabetic retinopathy (DR). The first of the aforementioned retinal dystrophies, RP, describes a wide umbrella of monogenic disorders characterized by the initial loss of rod photoreceptor function followed by a secondary loss of cone function.[30] Because cones more densely populate the central retina, the peripheral field of vision is lost as the rods die off leaving a small area of functional vision. Most RP cases demonstrate a secondary loss of cones following this rod death leaving the affected individual blind.[31] Findings from Punzo *et al.* include the realization that cone death is caused as a result of metabolite disruption and subsequent starvation. Many of the mutations in RP affect connecting cilia between the mitochondrially dense inner segment and high rate of turnover outer segments, likely affecting the lipid trafficking that delivers key energy substrates in a timely orchestrated manner.

The other major retinal dystrophies, DR and AMD are characterized by the rapid rate of mitochondrial dysfunction.[32] Many of the changes seen in the natural aging retina are exacerbated in both DR and AMD. Mitochondrial DNA repair mechanisms become impaired, possibly due to repeated oxidative stress leading to an accumulation of mutations and subsequent dysfunction.[16,33-39] DR is the leading cause of vision loss in mid-age working adults and is centered around vasculature abnormalities. It is likely caused by hyperglycemia-induced metabolic shifts that lead to mitochondrial degeneration and oxidative stress. Typical antioxidant agents that regulate levels of toxic oxygen radicals are suppressed in DR and hyperglycemic cell culture models however the mechanism of action remains yet to be properly explored.[40] These changes seemingly lead to impaired fatty acid β-oxidation and downstream ATP generation in DR. While RP primarily affects the peripheral vision, AMD is a disease of the central retina and macula and is the leading cause of vision loss in the aging population. Drusen accumulation is seen in the beginning stages of AMD and can progress towards RPE and photoreceptor degeneration. This accumulation of lipids is often seen in AMD progression[41,42] and is a potential risk factor for choroidal neovascularization.[43] As mentioned, mitochondrial degeneration and dysfunction are highly correlated with AMD progression, at an accelerated rate to that seen in the nondystrophic retina. VLDLR deficiencies discussed previously are associated with subretinal neovascularization and other key features of retinal angiomatous proliferation, a subtype of AMD,[44] possibly due to the mitochondrial dysfunction and inability to process the fatty acids generated by the phagocytized shed outer segment pieces.

Therapies to Renormalize Retinal Metabolism

As many of the underlying genetic mutations that lead to RP, AMD, and DR converge on similar downstream metabolic dysregulation, there has been a great interest in therapeutic strategies that upregulate protective antioxidant mechanisms and upregulate RPE-specific lipid metabolism in recent years. Caruso *et al.* elaborate on some of these proposed therapies.[45] Therein the authors discuss the estimated years of vision preserved as a result of their metabolic renormalization therapeutics based on the preservation seen in mouse models.[46] One therapy aimed at treating many underlying AMD mutations was explored by Iacovelli *et al.* wherein PGC-1α was overexpressed in a cell culture model. Findings include a stark increase in RPE-specific OXPHOS and fatty acid β-oxidation.[13] In the background of an underlying AMD mutation, this could upregulate functionality in the impaired mitochondrially dense RPE yet remains to be properly explored. Other mitochondria-focused degenerations in AMD explored by Karunadharma *et al.* raise the question of how protecting mitochondrial DNA in early disease stages could stop or revert disease progression as a whole.[39] Findings from Frank *et al.* indicate that the oxidative stress that accumulates lends itself toward AMD progression and that targeted therapeutics could ameliorate the stress, neovascularization, and disease progression as a whole.[47]

Metabolic reprogramming strategies are being investigated for a wide variety of neurodegenerative diseases including Huntington's disease. Tsunemi *et al.* showed that PGC-1α overexpression reduced protein aggregation and oxidative stress, indicating that a similar strategy could be feasible in RP and AMD and may prevent long-term Drusen formation altogether.[48] Upstream regulators of PGC-1α, including silence information regulator 1 and adenosine monophosphate-activated protein kinase (AMPK), were explored in a similar vein. Findings indicated that some neuroprotection was induced following the activation of these PGC-1α regulators raising the question of whether or not these therapeutic avenues could be translatable to the retina and RPE.[49,50] An alternative strategy to gene therapy was proposed by Brown *et al.*, hinging on small molecule compounds that upregulate genes or factors associated with mitochondrial biogenesis and related antioxidant properties, such as AMPK.[51] A different strategy for mitigating oxidative stress in RP was proposed by Gallenga

et al., where oxidative microglia are suppressed, breaking the harmful cycle of inflammation and degeneration caused by long-term oxidative stress.[52] Wu *et al.* explored another powerful antioxidant transcriptional factor, Nrf2, and found that overexpression specific to the RPE rescued morphology and ultimately paused degeneration in multiple mouse models of retinal degeneration.[53] Another interesting strategy for combating retinal dystrophies relies on the replacement of diseased RPE cells with healthy, autologous RPE to prevent secondary photoreceptor loss. Artero-Castro *et al.* explored this route, correcting Mer tyrosine kinase receptor deficient patient-derived stem cells and observed the restoration of wild-type protein expression and normal phagocytosis associated with RPE cells.[54] This work serves as a proof of concept for future precision medicine strategies.

Conclusion

The RPE heavily relies on lipid metabolism as an alternative means of ATP production rather than glycolysis, despite the high concentration of glucose being shuttled from the choroid to the photoreceptors. This lipid metabolism serves as a way to break down fatty acids, which can be deleterious in their abundance, a means of ATP production, to support the high energetic demands of the RPE, and as a means to upregulate the antioxidants that help mitigate long-term oxidative stress in the RPE. In many retinal degenerations, including RP, AMD, and DR, RPE mitochondrial function is heavily impaired, leading to the breakdown of the delicate metabolic coupling between the RPE and photoreceptors as well as other pathogenic complications. Interestingly enough, many of the changes observed in these retinal degenerations are also seen in the natural aging retina, albeit to a lesser, slower extent. Therapeutic avenues aimed at restoring health metabolic and antioxidant functionality appear to be on the cusp of a revolutionary breakthrough and will serve as an imprecision medicine, able to treat a wide variety of underlying conditions while facing significantly fewer hurdles than mutation-specific precision medicine strategies. Ultimately, the mechanism of mitochondrial functionality loss specific to the RPE and beyond demands further exploration for the proper development of targeted therapeutics.

Conflicts of interest
Stephen H. Tsang has received support from Abeona Therapeutics, Inc and Emendo. Stephen H. Tsang is the founder of Rejuvitas. The authors declare no non-financial competing interests.

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