

Glycolysis and glucose metabolism as a target for bioenergetic and neuronal protection in glaucoma

Pete A. Williams*, Robert J. Casson

Vision is arguably our most valued sense, yet approximately 340 million people globally suffer blindness or moderate visual impairment, highlighting the need to further develop and advance treatments for ophthalmic diseases. Glaucoma refers to a group of ocular disorders united by a clinically characteristic optic neuropathy with associated retinal ganglion cell loss. It is one of the most prevalent neurodegenerations globally, the leading cause of irreversible blindness, and affects ~80 million people worldwide (with an estimated further 40 million undiagnosed).

The major risk factors for glaucoma are advancing age, genetics, and high intraocular pressure (IOP). Current treatment strategies only target IOP management. Although under careful management, vision can usually be preserved, after 20 years, approximately 13% of individuals have progressed to bilateral blindness (Peters et al., 2013). Furthermore, increased life expectancies are making life-long retention of vision more challenging. Clinically translatable therapeutic strategies for glaucoma that do not target IOP lowering are urgently needed. Recently we demonstrated a strong neuroprotection in multiple cell and animal models of glaucoma-related injury focusing on pyruvate metabolism which was successfully translated into a Phase II randomized control clinical trial. Here we discuss the potential to target retinal ganglion cell metabolism via glycolysis/glucose metabolism for neuroprotection in glaucoma. An overview of these strategies is presented in **Figure 1**.

The intense energy requirements of the brain render it susceptible to bioenergetic failure. It is notable that bioenergetic dysfunction has been implicated in a variety of neurodegenerative diseases, at the level of glycolysis and oxidative phosphorylation. Parkinsonism is associated

with phosphoglycerate kinase deficiency, which catalyzes the first ATP-producing reaction in glycolysis (Tang, 2020). Brain regions with the highest levels of glycolysis in adulthood are those that have the highest susceptibility to Alzheimer's disease (Tang, 2020). Similarly, motor neuron disorder is also associated with bioenergetic abnormalities and aerobic glycolysis (Tang, 2020). Like other regions of the brain, retinal ganglion cells are also subject to metabolic compromise. Retinal ganglion cells are the output neuron of the retina and their axons make up the optic nerve. The retina is one of the most metabolically active tissues in the human body (Casson et al., 2021). Retinal ganglion cells are constantly under numerous bioenergetic-related stresses including blue light exposure, constant changes in vascular tone, limited glial support (comparative to other neurons in the central nervous system), having a large unmyelinated intraretinal portion of axon, as well as being tonically inactivated (expending increased energy during darkness/eye closure). Consequently, one can consider retinal ganglion cells to exist on a metabolic knife-edge, i.e. the level of energy dependence over the bioavailability of ATP and other essential metabolites.

To explore the role of retinal metabolism in glaucoma pathogenesis and treatment we performed RNA-sequencing and metabolomics to examine early degenerative events in DBA/2J mice (D2), a commonly used chronic pre-clinical model of glaucoma (Harder et al., 2020). In D2 mice in our colony, IOP raises from approximately 6 months of age with damage to the optic nerve evident from 10–10.5 months of age. We examined mice at 9 months of age where there is high IOP, but no detectable histological neurodegeneration. RNA-sequencing of FAC-sorted retinal ganglion cells demonstrated gene expression and pathway enrichment changes

that significantly impact pathways mediating the metabolism and transport of glucose and pyruvate. To add to this, we next performed untargeted metabolomics of whole retinas at the same time point. Pathway analyses of the altered metabolites showed enrichment in pathways related to glucose metabolism and oxidative stress corresponding to the changes we saw in sorted retinal ganglion cells. The largest change in a single metabolite at this time point was glucose (52-fold increase) likely reflecting altered glycolysis. We next used targeted assays to assess glycolytic metabolites that were not captured in our untargeted metabolomics protocols. These identified an IOP-dependent decline in retinal pyruvate levels prior to detectable optic nerve degeneration (Harder et al., 2020).

The energy needs of the retina are predominantly met via glycolysis and oxidative phosphorylation. Oxidative phosphorylation produces a significantly higher net yield of ~32 ATP compared to 2 ATP with glycolysis and is a key source of energy for retinal ganglion cells. However, recent evidence has clearly demonstrated that both pyruvate and lactate (glycolysis end products) serve as important energy sources in the retina. *In vitro* studies demonstrate that retinal ganglion cells can utilize either glucose, lactate, or pyruvate as an energy source (Vohra et al., 2019; Harder et al., 2020; Casson et al., 2021). Pyruvate is the main product generated from the breakdown of glucose by glycolysis and is converted to either acetyl-CoA or to lactate.

As both pyruvate and the essential neuronal metabolite nicotinamide dinucleotide (NAD) were low in retinas undergoing ocular hypertensive stress we hypothesized a NAD-dependent glycolytic block (pyruvate kinase) acting as a metabolic nexus leading to bioenergetic failure and retinal ganglion cell compromise (Williams et al., 2017; Harder et al., 2020; Tribble et al., 2021). To definitively test this, we performed long-term oral supplementation with pyruvate in D2 mice. This treatment significantly protected against IOP-induced metabolic changes, as well as reduced optic nerve degeneration, axon transport disruption, and improved retinal ganglion cell visual function. To support these data, we also assessed pyruvate treatment in a rat laser photocoagulation model of sub-acute ocular hypertension and in mouse retinal axotomy explants. Pyruvate was strongly neuroprotective in these settings. We next combined a treatment of low-dose nicotinamide (an upstream precursor to NAD in the salvage pathway) and pyruvate. This treatment increased retinal ganglion cell axon survival greater than either treatment alone. Nicotinamide and pyruvate are both important for glycolytic regulation and we observed a synergistic effect using these two metabolites in combination to improve glycolytic capacity (Williams et al., 2017; Harder et al., 2020).

Lactate is also an important energy source for neurons. The monocarboxylate transporter 1 (MCT1) allows the transport of lactate through the blood-brain barrier as well as the inner and outer blood-retina barrier. Supporting a hypothesis in which pyruvate and lactate are important metabolites to retinal ganglion cells, both mitochondrial pyruvate carriers 1 and 2 (MPC1 and MPC2) have been demonstrated to be present at high levels in retinal ganglion cells

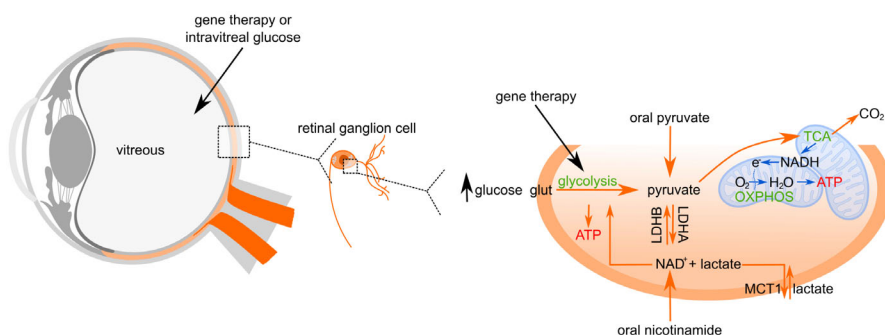


Figure 1 | Manipulating glucose metabolism in glaucoma.

Retinal ganglion cells derive adenosine triphosphate (ATP) from glycolysis and oxidative phosphorylation (OXPHOS). Glucose enters retinal ganglion cells via glucose transporters (glut), and the vitreous can serve as a reservoir. Nicotinamide dinucleotide (NAD) is a critical redox molecule required for glycolysis and formed by the tricarboxylic acid cycle (TCA) as an electron donor and can be derived from oral nicotinamide. Pyruvate is converted to lactate in a reversible reaction catalyzed by the isoenzymes lactate dehydrogenase A (LDHA) and LDHB. MCT1: Monocarboxylate transporter 1. Created with Inkscape (<https://inkscape.org/>).

across a number of species (Chidlow et al., 2005; Harder et al., 2020). Lactate and pyruvate are strongly neuroprotective to retinal ganglion cells in culture, including under glucose deprivation (Harder et al., 2020). Studies utilizing D2 mice and a mouse model of ocular hypertension have demonstrated reduced levels of L-lactate and a reduction in MCT2 receptor protein levels with MCT2 receptor overexpression demonstrated to be neuroprotective (Harun-Or-Rashid et al., 2020). Supporting this, pharmacological inhibition of MCTs blocks the neuroprotective effects of pyruvate on cultured retinal ganglion cells. Collectively, these experiments provide strong evidence for the role for glycolysis as a target for neuroprotection in glaucoma.

Pyruvate is an ideal treatment to test for clinical use, with a long history and good safety profiles in humans (with the only major side effect being diarrhea, typically when taken at greater than 30 g/day). To increase the neuroprotective capacity of pyruvate we tested a combination therapy of pyruvate and nicotinamide. Nicotinamide has been demonstrated to be strongly neuroprotective at high doses (Williams et al., 2017; Tribble et al., 2021). Combination therapy of pyruvate and nicotinamide (at lower doses than used individually) lowered the risk of optic nerve degeneration in D2 mice by ~2.6 fold, more than either treatment alone.

But how do we translate this increasing knowledge of glycolytic susceptibility in glaucoma animal models into clinically available neuroprotective strategies for glaucoma? Our first clues come from a study from Casson and colleagues who performed a double-blind randomized study testing 50% glucose eye drops *versus* saline (Casson et al., 2014). In this study, primary open-angle glaucoma patients were randomly allocated saline or 50% glucose eye drops every 5 minutes for 60 minutes (Casson et al., 2014). Glucose successfully reached the vitreous of pseudophakic individuals and glucose eye drops significantly improved contrast sensitivity over control. These data support an earlier study in which elevating intravitreal glucose levels provides neuroprotection in a rat model of retinal ischemia and, together, supports the notion of bioenergetically compromised retinal ganglion cells as a target for recovery.

Given these exciting short-term findings in glaucoma patients with glucose eye drops, and our results demonstrating a strong neuroprotection with pyruvate and nicotinamide in glaucoma animal models, we next set off to apply this in a clinical trial setting. To test pyruvate and nicotinamide in combination (our most neuroprotective setting in glaucoma models), De Moraes et al. (2022) performed a randomized Phase II clinical trial testing pyruvate 3 g/day plus nicotinamide 3 g/day in primary open-angle glaucoma patients. This study demonstrated improved visual function (pattern standard deviation (visual field)) in existing glaucoma patients versus placebo control (average 2 months follow-up) in addition to high adherence rates (only 1 patient withdrawn and no reported adverse effects). If we take the pre-clinical and clinical data in concert, then this paints an attractive narrative where energy supplementation not only provides neuroprotection, but also provides neurorecovery with improved visual function. Importantly, these approaches are directly therapeutically

approachable and, if combined with IOP-lowering therapies – the current gold standard in glaucoma – represent a powerful therapeutic strategy for human glaucoma.

The mechanism underpinning intraocular pressure-associated glaucomatous axonal degeneration remains unclear. However, there is considerable evidence that vascular insufficiency at the level of the optic nerve head and the retina plays a role. This is particularly prominent in normal-tension glaucoma, one could speculate that bioenergetic strategies would be particularly suited to this form of glaucoma. However, targeting glycolysis at the level of the retinal ganglion cells presents challenges. Persistently, elevating vitreous glucose levels, for example, by some method of local administration may cause profound diabetic retinopathy (whether elevated vitreal glucose in the absence of hyperglycemia would actually cause a retinal microangiopathy is untested). Oral supplements may need to be in supraphysiological concentrations to reach therapeutic levels, and gene therapy manipulation of glycolytic enzymes may have unexpected adverse consequences. Future research in retinal bioenergetics will encompass benchwork aiming to better understand energy metabolism at the cell-specific level and translation to clinical trials. Interrogating *in vivo* metabolism is challenging and will benefit from emerging technologies such as hyperspectral imaging. A better understanding of what retinal ganglion cells ‘like to eat’ will guide optimal translation. In addition, the field is likely to benefit from advances in gene therapy, CRISPR approaches, and targeted manipulation of glycolytic enzymes. Alternative methods of manipulating metabolism with visible light are being investigated. Clinical neuroprotection research in glaucoma is in its infancy but is benefiting from collaborative strategies and sophisticated trial designs.

Recent clinical and pre-clinical evidence suggests that retinal ganglion cells have the capacity to recover function and is supported by evidence that initial IOP-lowering leads to a transient increase or recovery of functional vision. Together, this has introduced the concept of the “coma in glaucoma”, the idea that retinal ganglion cells undergoing neurodegenerative cascades are part of a heterogeneous population of dead, dying, stressed, and alive cells – a critical plastic state that is amenable to functional recovery if the right conditions are met (Fry et al., 2018). If energy failure is part of the pathogenesis then it is logical that bioenergetic strategies may support neurorecovery and, in the chronic situation, support neuroprotection in a manner analogous to intraocular pressure reduction.

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