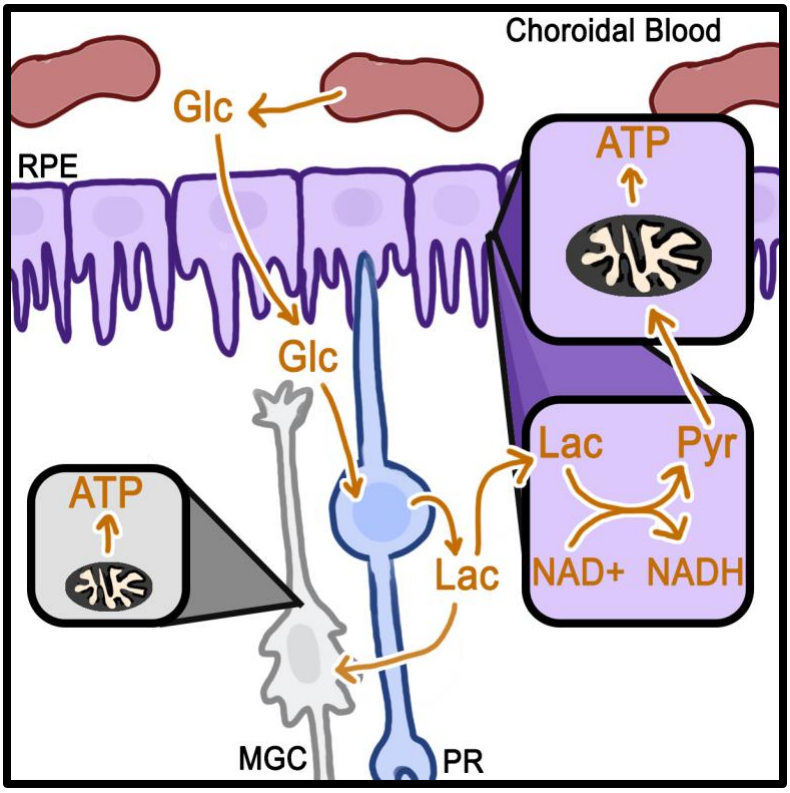
Graphical Abstract:



The Basics: This paper involved the nature of energy metabolism in the retina and in retinal pigment epithelium (RPE)[1]. Previous research had determined that retinas and tumors rely mostly on aerobic glycolysis, and specifically that photoreceptors (PRs) in outer retina are the site of aerobic glycolysis.

In PRs, genetic changes targeting the glycolytic pathway, such as the enzyme lactate dehydrogenase, decrease the lactate production of photoreceptors. PRs convert ~80% of glucose to lactate through glycolysis and the process supports multiple metabolic demands within the cell[2]. Furthermore, it is known that making the RPE more glycolytic causes neighboring photoreceptors to degenerate while making PRs more glycolytic is beneficial. In response to hypoxia, RPE reduces oxidative phosphorylation and TCA cycle while increasing glycolysis. Glucose transporter GLUT1 is also upregulated, leading to increased glucose intake, and PR degeneration[3].

While RPE and PR responses to increased glycolysis was previously known, the relationship between RPE and PR metabolism was unknown. This paper elucidates this relationship to better the understanding of the cellular environment. The experiments in the paper used confocal microscopy to image tissues, immunoblotting, experiments with mice using oral gavages, and gas chromatography mass spectrometry (GC-MS).

Through their work they found that glucose is transported through the RPE cells and PRs primarily take up glucose and convert it to lactate. This lactate is fuel for neighboring cells and impairs glucose metabolism by the RPE cells to increase glucose availability for PR cells. Increased understanding about the metabolic ecosystem between RPE and PR cells create a foundation for better treatments for retinal diseases, along with further research about the interactions between different types of differentiated cells.

Key Findings: The overall goal of the paper was to determine a relationship between RPE and PR cell metabolism. This was mainly done by observing and comparing the paths of glucose and lactate through RPE and PR cells.

Initially, immunoblot analysis was used to confirm that GLUT1 is present and membrane-associated in both mouse retina and RPE. Confocal microscopy was used to determine spatial distribution of GLUT1 in the retina in comparison to other elements such as Müller glial cells (MGCs) and rods. The overlap between GLUT1 and PRs led the conclusion that PRs are able to uptake glucose released from the apical side of the RPE.

Due to overlap between the MGCs and GLUT1, MGC uptake of glucose was tested. Oral gavage was used to introduce a fluorescent derivative of 2-deoxy glucose (2-NBDG) to mice. After incubation of the 2-NBDG, the mice were euthanized, and retinas harvested and imaged using confocal microscopy. The 2-NBDG fluorescence was spatially compared to the fluorescence of MGCs in mice and zebrafish. The comparison showed that there was no overlap between the 2-NBDG and MGCs, indicating that MGCs did not uptake glucose.

Comparison of the of mouse retina (mRetina) and cultured human fetal RPE (hfRPE) treated with ^{13}C glucose was used to compare incorporation of ^{13}C into glycolytic and TCA cycle intermediates. Glycolytic metabolites were more abundant in mRetina and TCA cycle intermediates ware more abundant in hfRPE. ^{13}C was incorporated into lactate, a product of glycolysis, eight times faster in mRetina than hfRPE. These data showed mRetina and hfRPE use glucose differently and that mRetina has higher glycolytic activity and efficiency.

Experiments incubating hfRPE in ^{13}C lactate showed citrate, a TCA cycle metabolite, is formed quickly from lactate, which indicated hfRPE can use lactate as fuel.

The experimenters then hypothesized that lactate could suppress glucose consumption by reducing NAD+, a required reactant of glycolysis, in RPE. NADH fluorescence of hfRPE in a solution of glucose and lactate confirmed that the addition of lactate increases NADH fluorescence, whereas adding pyruvate decreased the fluorescence. This indicates lactate depleted NAD+ in RPE and suppressed glycolysis. Glyceraldehyde-3-phosphate (GAP), which normally participates in the NAD+ requiring glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reaction in glycolysis, accumulated because NAD+ became unavailable, and downstream product concentrations decreased. Furthermore, after incubating hfRPE in $_{13}$C glucose with or without unlabeled lactate, GC-MS results indicated unlabeled lactate was used to create TCA cycle metabolites.

Finally, hfRPE grown on transwell filters was used to observe glucose transport. $_{13}$C glucose was added to the basolateral side, which normally faces the choroidal blood supply. The apical side had either 10mM unlabeled lactate or no lactate. GC-MS was used to quantify $_{13}$C glucose on apical side after some time and results displayed that the presence of lactate on the apical side increased $_{13}$C glucose accumulation. $_{13}$C pyruvate and $_{13}$C lactate accumulation is also decreased, which further indicated that the glucose is not used for glycolysis in hfRPE.

All these experiments logically stemmed from one another and used both negative and positive controls. For the experiments in which TCA metabolites were measured to compare glycolytic activity of hfRPE with mRetina, controls for cells depending either completely on glycolysis or oxidative phosphorylation would have contributed useful standards for the data. Both controls would likely be genetically modified, although the negative control may be more difficult to create because most cells depend on glycolysis to various extents.

Conclusions: While both RPE and PR cells have the ability to undergo glycolysis, glucose is generally transported from the choroidal blood to the apical side of the RPE and to the PR cells. PRs produce lactate from the glucose using glycolysis, which functions as fuel for neighboring cells, including MGCs and the RPE. The produced lactate also suppresses glycolysis in the RPE to ensure sufficient supply of glucose for the PRs. In part, this suppression is a result of the lactate reducing NAD+, an essential reactant for the glycolytic enzyme GAPDH, to NADH in RPE, which is then used in oxidative phosphorylation.

Future Directions: This research can be used to support therapeutic research of retinal degeneration. This research explains some reasons increased glycolytic activity in RPE would be damaging to PRs while increased glycolytic activity in PRs is beneficial. By understanding the role of lactate in the system, research could be done with RPE cells of higher sensitivity to lactate to combat failing PRs.

Another interesting link to explore would be how diabetes affects the metabolic ecosystem of the RPE and PRs. Diabetic retinopathy is related to high blood sugar, which causes damage to the retina. However, the system from this paper indicates that high blood sugar will lead to the PRs receiving more glucose and, as such, suppressing the RPE's glycolytic activity further. It would be interesting to explore the link between the PRs, which should be more robust, and the retinal degeneration.

Beyond therapeutic research, more research can be done on how the accumulation of GAP in the RPE is dealt with by the cells. Accumulation of a product infinitely would likely lead to some type of toxic effect in the cell or, at the very least, take up space in a cell. Understanding GAP in the RPE cells would elucidate the other processes taking place in the cells.

Works Cited:

1. Kanow, M. A. *et al.* Biochemical adaptations of the retina and retinal pigment epithelium support a metabolic ecosystem in the vertebrate eye. *Elife* **6**, (2017).

2. Chinchore, Y., Begaj, T., Wu, D., Drokhlyansky, E. & Cepko, C. L. Glycolytic reliance promotes anabolism in photoreceptors. *Elife* **6**, (2017).

3. Kurihara, T. *et al.* Hypoxia-induced metabolic stress in retinal pigment epithelial cells is sufficient to induce photoreceptor degeneration. *Elife* **5**, (2016).