Utilizing Fluorescent Lifetime Imaging (FLIM) to Investigate Beta Cell Metabolism

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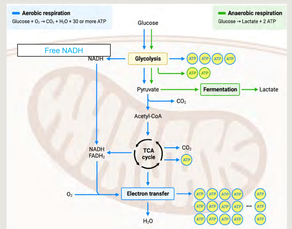
Abstract

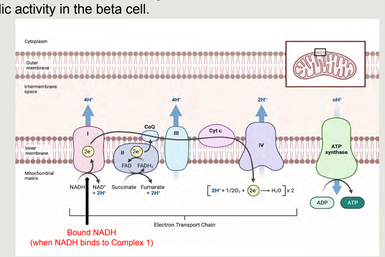
The pancreas contains groups of cells called pancreatic islets that play a critical role in controlling blood sugar levels in the body. Islets contain endocrine cells such as beta cells, which release insulin to reduce blood glucose. Beta cell loss, stress, and dysfunction can cause hyperglycemia and eventually diabetes. The goal of this project is to study the changes in beta cell metabolism from low to high glucose in the context of the whole islet and surrounding pancreatic tissue by using Fluorescent Lifetime Imaging (FLIM). This will give us insight into the complex cellular processes that direct insulin secretion. First, we obtained slices or isolated islets from the pancreas of a mouse. We placed the slices or isolated islets in low glucose and imaged them using a FLIM microscope. Then, we exposed the slices or isolated islets to high glucose and imaged them at each 10-minute interval for 90 minutes. Next, we developed a custom FLIM image processing workflow in Matlab to produce phasor plots and pseudo-colored %bound NADH images from microscope images. The data reveals that beta cells undergo more glycolytic activity in low glucose as the pseudo-colored image is predominantly blue, indicating a lower percentage of bound NADH. Beta cells undergo more oxidative phosphorylation (OXPHOS) activity in high glucose as the pseudo-colored image is predominantly yellow and green, indicating a higher percentage of bound NADH. Slices were found to have a more gradual increase in metabolic activity compared to isolated islets. Our work with FLIM has helped us further our understanding of beta cell metabolism under high and low glucose conditions. Healthy beta cells respond to high levels of glucose by undergoing more OXPHOS activity to produce more energy for insulin secretion. The next step is to use FLIM to study the different beta cell subpopulations within an islet.

Background

Metabolism: High vs. Low Glucose

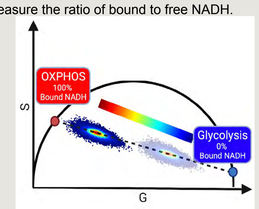
The regulation of glycolysis and oxidative phosphorylation is a key factor in beta cell function. In low glucose, beta cells have a lower ratio of OXPHOS to glycolytic activity. In high glucose, healthy beta cells have a higher ratio of OXPHOS to glycolytic activity to maximize energy output for insulin secretion. However, for diabetic beta cells, the ratio of OXPHOS to glycolytic activity remains low under high glucose conditions. To investigate the metabolic state of beta cells, we look at free NADH and bound NADH. Free NADH is produced during glycolysis while NADH binds to complexes along the Electron Transport Chain during OXPHOS. Thus, the ratio of bound NADH to free NADH indicates the level of metabolic activity in the beta cell.





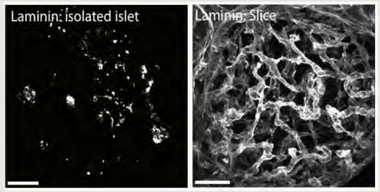
What is FLIM?

A fluorescent image is formed when a laser hits and excites the fluorophore. FLIM measures the lifetime of the fluorophore from each pixel and plots it on a phasor plot. A color map can be used to create a lifetime mask that pseudo-colors the corresponding pixels on the fluorescent image. Since the lifetime of free NADH is 0.4ns and the lifetime of bound NADH is 1.0 to 4.0ns, FLIM can be used to measure the ratio of bound to free NADH.



Islets vs. Slices:

Slices preserve beta cell structure and are more biologically representative of the processes that happen *in vivo*. The perfusion solution used to isolate islets from the pancreas destroys blood vessels and other connections the islets have with their original endogenous environment.



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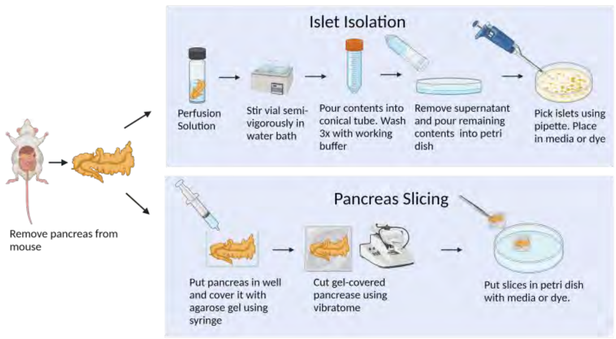
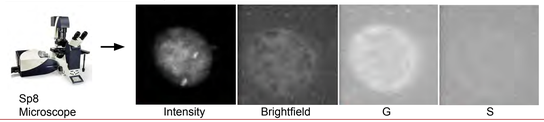
Methods

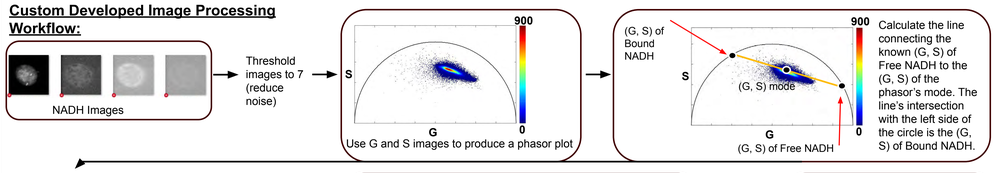


Data Input: NADH Images of Islets/Slices

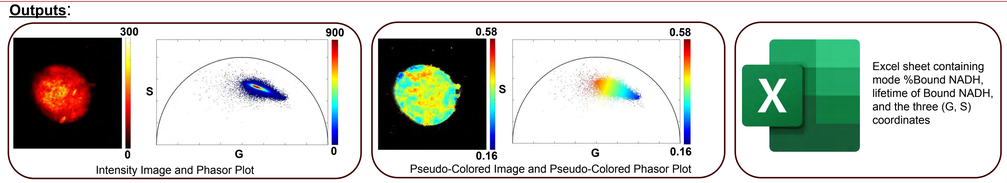


Four types of NADH images were taken at each 10-minute interval from 0 minutes to 90 minutes. Initially, islets were placed in low glucose. High glucose was added after the 0-minute mark.

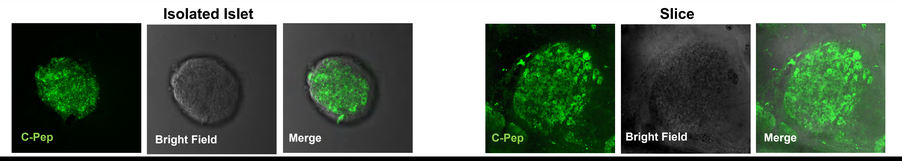
Custom Developed Image Processing Workflow:



Outputs:



Visualizing Beta Cells



Results: Isolated Islet

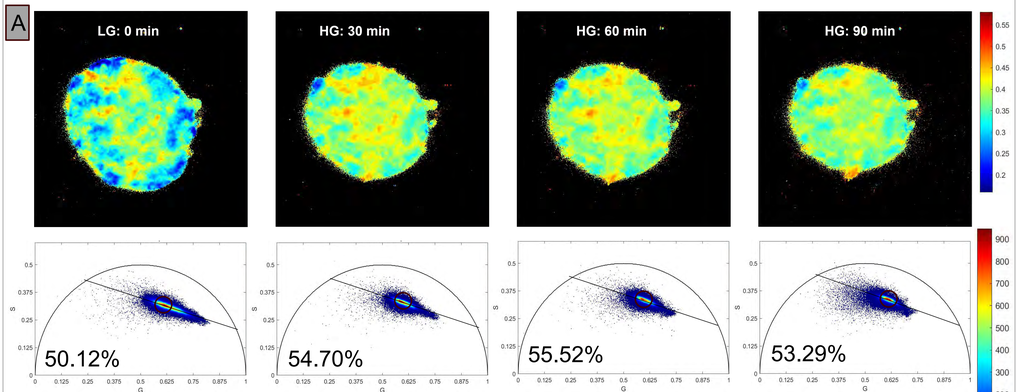


Figure 1: (**A**) The islets are pseudo-colored based on %bound NADH per pixel and the mode %bound NADH is shown in the bottom left corner of the phasor. Beta cells undergo more glycolytic activity in low glucose, so the pseudo-colored islet is predominantly blue, indicating low %bound NADH. In high glucose, beta cells undergo more OXPHOS activity so the pseudo-colored islet is predominantly yellow and green, indicating a higher %bound NADH. (**B**) The graph demonstrates how the mode %bound NADH of the islet increases suddenly when the islet is exposed to high glucose as the beta cells become more metabolically active. (**C**) The (G,S) coordinate of the phasor's mode is plotted over time to demonstrate how the phasor shifts suddenly to the left after high glucose is added, marking an increase in %bound NADH.

Results (Continued): Slice

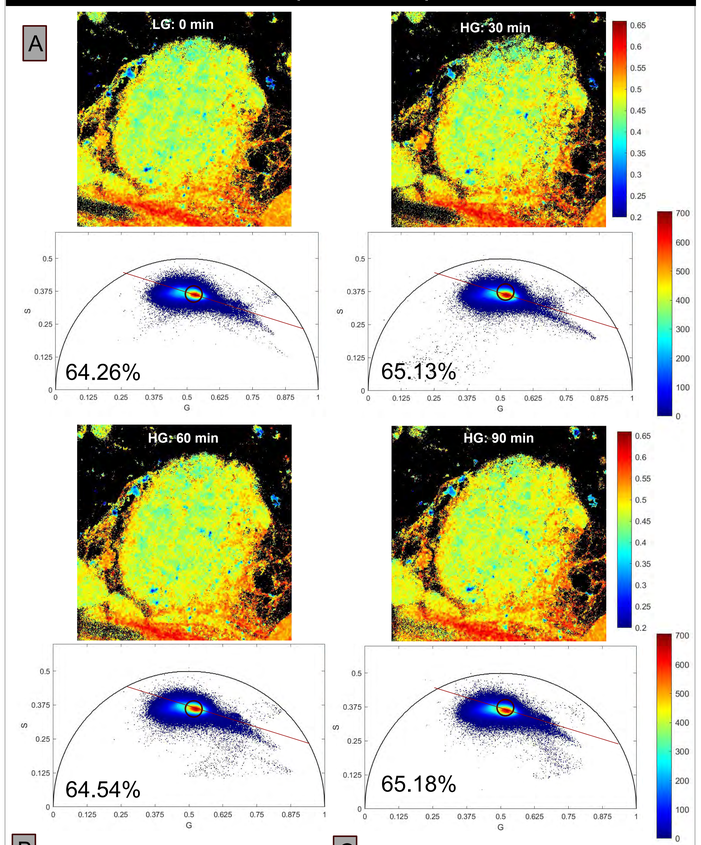


Figure 2: (A) The slices are pseudo-colored based on %bound NADH and the mode %bound NADH is shown in the bottom left corner of the phasor. The observed increase in metabolic activity from low glucose to high glucose is more gradual than in the isolated islet as regions of the islet slowly change from green to yellow to red.**(B)** The graph demonstrates how the mode %bound NADH of the slice increases gradually when the slice is exposed to high glucose. **(C)** The (G,S) of the phasor's mode is plotted over time to demonstrate how the phasor moves slowly to the left after high glucose is added as the beta cells become more metabolically active.

Conclusion

We imaged isolated islets and slices using FLIM to investigate beta cell metabolism from low glucose to high glucose conditions. In high glucose, beta cells are more metabolically active with higher ratios of OXPHOS to glycolytic activity than in low glucose. We found that the metabolic activity of slices in high glucose gradually increases over time while the metabolic activity of isolate islets in high glucose increases dramatically before staying relatively constant. Our work with FLIM helps to visualize the shifts in beta cell metabolism that direct insulin secretion.

More data is required to confirm the difference in metabolic behavior between slices and isolated islets. Furthermore, the next step of our project is to use FLIM to investigate beta cell heterogeneity. Hub cells, a subpopulation of beta cells in an islet, direct follower beta cells to secrete insulin. Regions of an islet that exhibit early OXPHOS activity in high glucose could be evidence of hubs recruiting surrounding beta cells in a wave pattern of activation.

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