Deep Learning-Based Image Segmentation of Mouse Adult Primary Cardiomyocytes Predicts Viability, Bioenergetic Status & Nutrient Sensitivity

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Background

Recent advancements in culture media for human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) include addition of fatty acids to promote adult-like metabolic maturation. This media improves the relevance of human iPSC-CMs as a model to characterize metabolic disorders. However, adult primary cardiomyocytes (adult CMs), isolated from the heart, retain a structural fidelity that human iPSC-CMs lack and could be a more relevant model of cell metabolism, but human adult CMs are generally unavailable. Mouse adult CMs can be readily isolated but require improved characterization methods to understand their relevance as a model of metabolism. The distinct rod-shape of adult CMs provides an opportunity to track cell morphology and facilitate improvements to characterization methods. Therefore, in this study we implemented deep learning-based segmentation of WT mouse adult CM imaging to track morphology and compare bioenergetic methods with those optimized for WT human iPSC-CMs.

Methods

We used Seahorse XFe96 technology to measure oxygen consumption and extracellular acidification rates (OCR & ECAR), and calculate mitoATP rate, glycoATP rate, and spare respiratory capacity (SRC). We also implemented deep learning-based segmentation of live WT mouse adult CM imaging to classify morphology (Cellpose v2.2.2, Cellprofiler v4.2.6). Assays were run in Dulbecco's Modified Eagle Medium (XF DMEM) buffer with glucose (10 mM), glutamine (2 mM), carnitine (0.5 mM), palmitate-BSA (100 µM) and pyruvate (1 mM), unless otherwise specified. Seahorse metabolic modulators were used at the concentrations shown in the figures. WT human iPSC-CMs were cultured in fatty acid (FA)-enriched metabolic maturation culture media between 8-14 days before Seahorse assays. Adult CM isolation & assay buffers include a myosin ATPase inhibitor (3µM mavacamten) to prevent terminal contracture. Data shown are average ± SD. Significance via ANOVA.

Wild Type (WT) Human iPSC-CMs Exhibit Fuel Flexibility but Prefer Fatty Acid Oxidation (FAO)

With palmitate-BSA added to Seahorse assay buffer, WT human iPSC-CMs were relatively insensitive to inhibition of pyruvate and glutamine mitochondrial consumption, while gaining sensitivity to inhibition of fatty acid oxidation. This indicates WT human iPSC-CMs have fuel flexibility but adopt fatty-acid fueled bioenergetics even when alternative fuels are available.

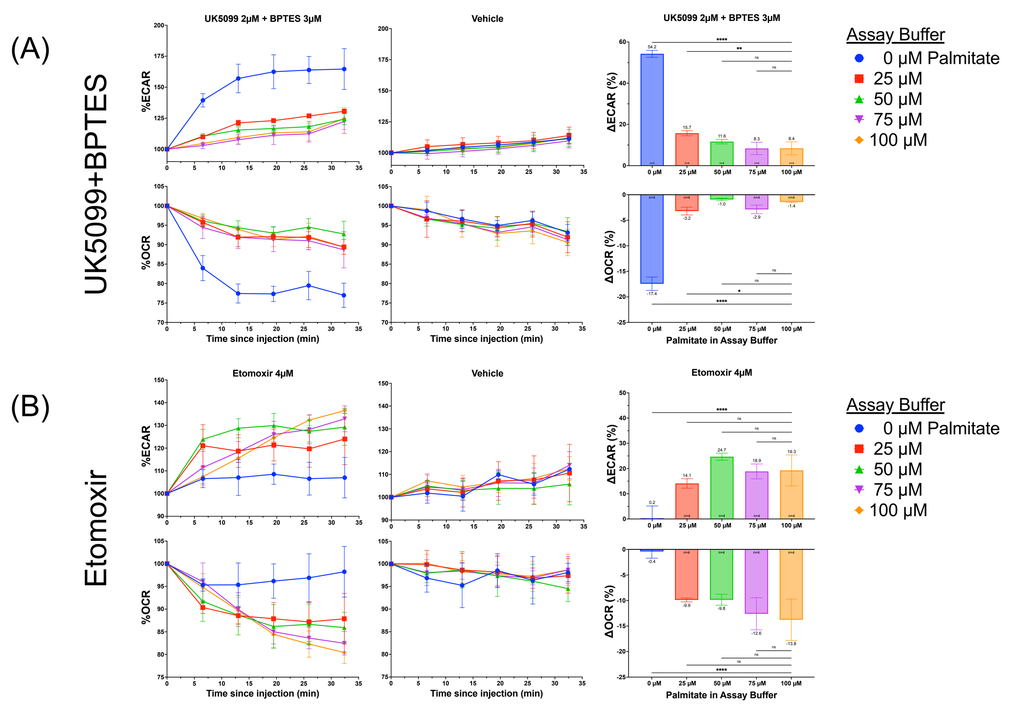

Figure 1. (A) Fatty acid enrichment (palmitate-BSA) of Seahorse assay buffer reduces sensitivity to the combined inhibition of Mitochondrial pyruvate carrier (MPC/UK5099) & Glutaminase (GLS1/BPTES). **(B)** 100µM palmitate-BSA maximizes the effect of Carnitine palmitoyltransferase 1 (CPT1/etomoxir) inhibition, indicating long-chain fatty acid oxidation (FAO) is preferred in the presence of palmitate.

WT Human iPSC-CMs in Physiological Pyruvate Reveal FAO Dependence

Basal bioenergetics of WT human iPSC-CMs cultured in FA rich media are predominately mitochondrial and sensitive to inhibition of FAO. However, spare respiratory capacity is only sensitive to FAO inhibition in assay buffers with pyruvate reduced to physiological concentrations.

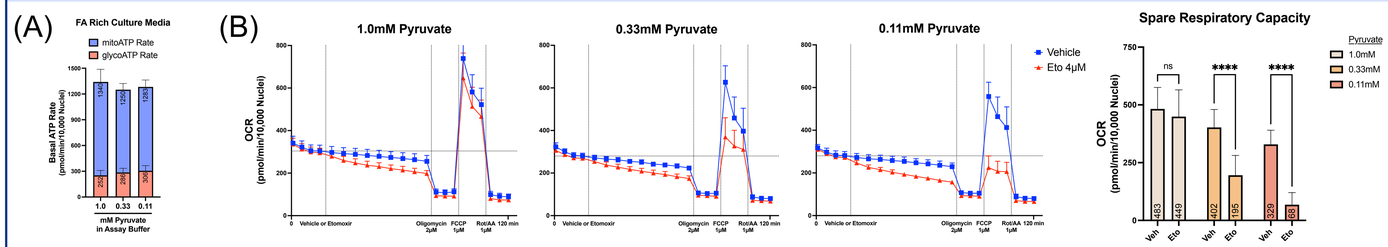

Figure 2. (A) FA-enriched metabolic maturation culture media results in high oxidative phosphorylation (mitoATP Rate) and low glycoATP Rate. **(B)** Reducing pyruvate in Seahorse assay buffer containing palmitate-BSA (100 µM) results in spare respiratory capacity that is sensitive to etomoxir inhibition, indicating long-chain FAO in mitochondria can be the dominant source for maximal ATP production when pyruvate in Seahorse buffer is reduced to physiological levels (plasma pyruvate = 0.03-0.26mM; *Rao 2021*)

WT Mouse Adult CMs Require Pyruvate to Maintain Viability

Basal OCR and spare respiratory capacity of isolated and non-paced WT mouse adult CMs were insensitive to FAO inhibition in the presence of pyruvate and glutamine, suggesting a possibly 'arrested' state of bioenergetics in the absence of cellular contractions. Removing pyruvate to force cells to rely on FAO was unsuccessful due to lack of viability.

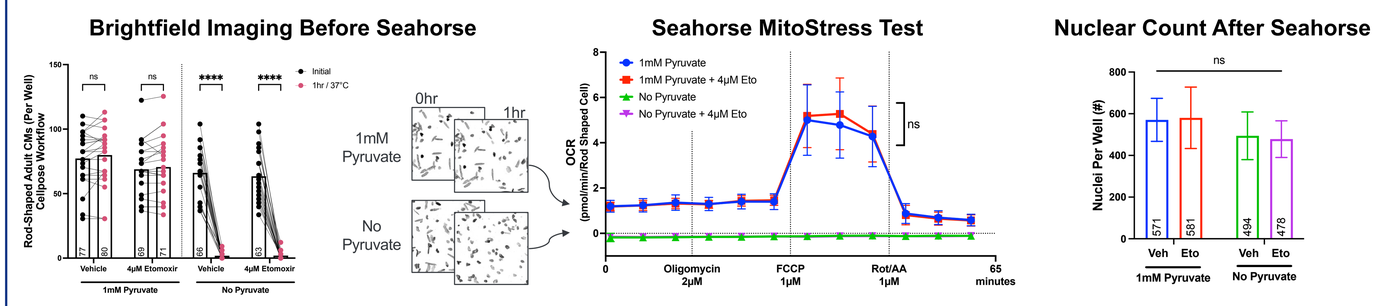

Figure 3. WT mouse adult CM morphology was assessed by brightfield imaging in assay buffer for 1hr before Seahorse MitoStress Test. Rod-shaped cell count before Seahorse assay, but not nuclear count after Seahorse assay, predicted bioenergetic status. Without pyruvate in the assay buffer, adult CMs lost their rod morphology and had no measurable OCR or ECAR (ECAR data not shown). Therefore, rod-shaped morphology is an indicator of viability. Spare respiratory capacity of adult CMs was not sensitive to etomoxir in 1mM pyruvate. No oligomycin response was observed, as previously described for adult CMs (*Readnower 2012)*.

Deep Learning Segmentation Predicts Viability of WT Mouse Adult CMs

Live-cell brightfield imaging of WT mouse adult CMs and deep learning-based segmentation were used to track morphology and screen for optimal pyruvate in bioenergetic assay. WT mouse adult CMs were acutely sensitive to even moderately reduced levels of pyruvate, with morphology changes indicating no viability with reduced pyruvate levels.

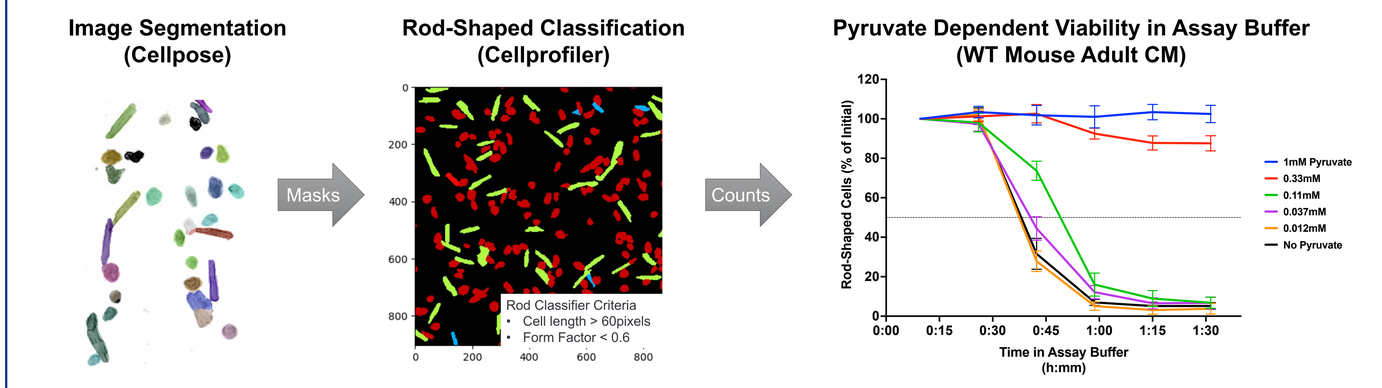

Figure 4. Pyruvate-dependent viability of WT mouse adult CMs was assessed in Seahorse assay buffer using deep learning-based segmentation of live-cell brightfield images and a rod classifier to generate a timecourse of morphology, which predicts viability. Low or no pyruvate led to cell death for the majority of rod-shaped WT mouse adult CMs in a timespan shorter than the duration of a Seahorse assay. WT mouse adult CMs maintained rod morphology only at 1mM or 0.33mM pyruvate concentrations in Seahorse assay buffer. The segmentation and rod classifier workflow was comparable to manually counted data (not shown; R^2=0.95 compared to manual count results; >2000 rod-shaped cells counted across all timepoints).

Summary

- Relevant cell culture milieus provide an enhanced WT human iPSC-CM platform for characterizing disease models and metabolic disorders.
- The distinct rod-shape of WT mouse adult CMs enabled deep learning-based segmentation of imaging to track WT mouse adult CM morphology
- Combining orthogonal measurements of morphology and bioenergetics reveals *in vitro* differences between WT mouse adult CMs and WT human iPSC-CMs.
- Isolated WT mouse adult CMs require further optimization of assay media viability and exploration of stimulation-based contraction to reveal *in vivo*-like bioenergetics.

References: Readnower, et al. (2012). *Physiol Genomics*. 44., Feyen, et al. (2020). *Cell Rep*. 32., Rao, et al. (2021). *J Biol Chem*. 297., Stirling, et al. (2021). *BMC Bioinformatics*. 22., Stringer, et al. (2021). *Nat Methods*. 18., Angelini, et al. (2022). *STAR Protoc*. 3.