Recovery From Ischemia-Reperfusion Injury Is Metabolic Substrate-Dependent

Sergiy M. Nadtochiy, PhD and Paul S. Brookes, PhD, FAHA

Contacts: sergiy_nadtochiy@urmc.rochester.edu; paul_brookes@urmc.rochester.edu

Department of Anesthesiology, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester NY 14620, www.psblab.org

Aim

The ability of the heart to metabolize specific substrates could impact the outcome of pathological insults, such as ischemia-reperfusion (IR) injury. The aim of this study was to develop a system whereby metabolism of adult mouse cardiomyocytes (AMC) supplied with different fuels, could be measured in real time during simulated IR injury.

Methods

Adult mouse cardiomyocytes (AMC) were isolated as previously described [1] and were plated on laminin-coated V7-PS plates (Seahorse Bioscience, North Billerica, MA). A previously developed method for in-vitro IR injury using a Seahorse XF24 metabolic flux analyzer [2], was adopted for AMC (Figure 1). Briefly, initial argon flow delivered a drop in pO_2 to <10 mmHg, and then simulated ischemia was initiated by holding down the plungers; cells consumed all available O_2 within the 7 µl chamber, and entered an ischemic state. Then, raising the plungers and flushing plate with room air simulated reperfusion.

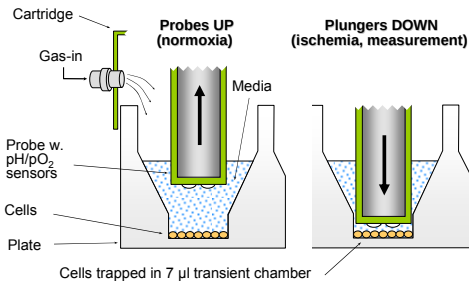


Figure 1. Adaptation of XF24 for in-vitro simulated IR model.

ACM were supplied with either glucose (GLU, 5mM) plus etomoxir (20µM, fatty acid inhibitor); palmitate/fat-free BSA (FAT, 100µM) plus 2-deoxy-D-glucose (10mM, inhibitor of glycolysis); or GLU+FAT (no etomoxir or 2-deoxy-D-glucose). For simulated IR, AMC were exposed to 60 min. ischemia, followed by 60 min. reperfusion.

Pre- and post-ischemic oxygen consumption rate (OCR, mitochondrial respiratory activity) and extracellular acidification rate (ECAR, glycolytic activity) were measured to investigate metabolic changes. At the end of reperfusion LDH release was measured according to the manufacturer's instructions (Roche, Indianapolis, IN) and normalized to total LDH.

Results

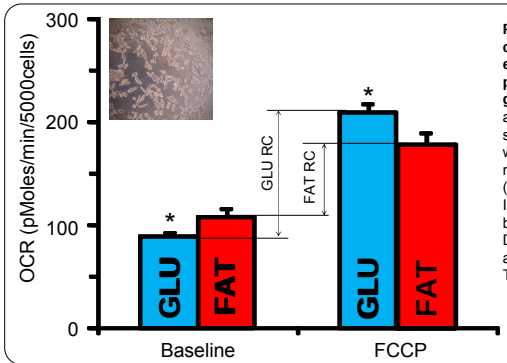
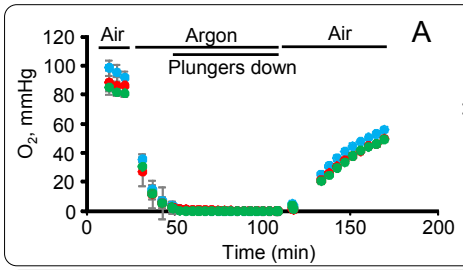


Figure 2. Basal and uncoupled oxygen consumption rate (OCR) in AMC fed with either glucose (GLU) plus etomoxir or palmitate/fat-free BSA (FAT) plus 2-deoxy-D-glucose. OCR was measured at baseline and after FCCP (500nM) injection. Uncoupler FCCP stimulated mitochondrial respiratory chain, which caused increase of OCR. Metabolic reserve capacity (RC) is indicated with arrows (RC = OCR FCCP- OCR Baseline).
Insert: picture of AMC on the V7-PS plate before measurements.
Data from three independent plates are shown as means ± S.E., n = 3. *, p < 0.05 versus FAT, T-TEST.

Results



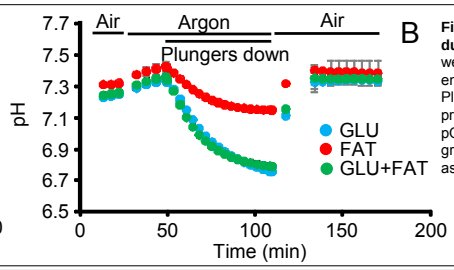
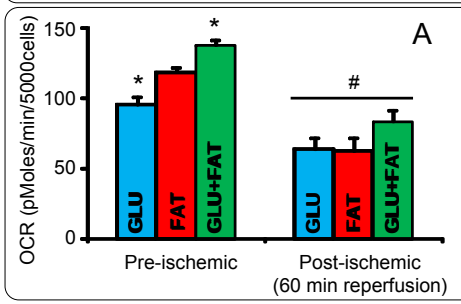


Figure 3. Typical XF24 pO_2 and pH traces during simulated IR. pO_2 (A) and pH (B) were measured by fluorescent probes embedded in the plunger in each well. Plungers were lowered for 60 min. in the presence of argon flow to simulate ischemia. pO_2 and pH from all wells within the same groups were averaged and data are shown as means ± S.D., for a single plate.



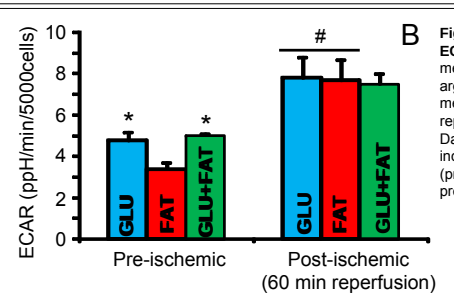


Figure 4. Pre- and post-ischemic OCR and ECAR. Pre-ischemic OCR and ECAR measurements were taken prior to beginning argon flow over the plate. Post-ischemic measurements were taken at 60th min of reperfusion.
Data are the means ± S.E., n = 5 independent plates *, p < 0.05 versus FAT (pre-ischemic), ANOVA. #, p < 0.05 versus pre-ischemic, ANOVA

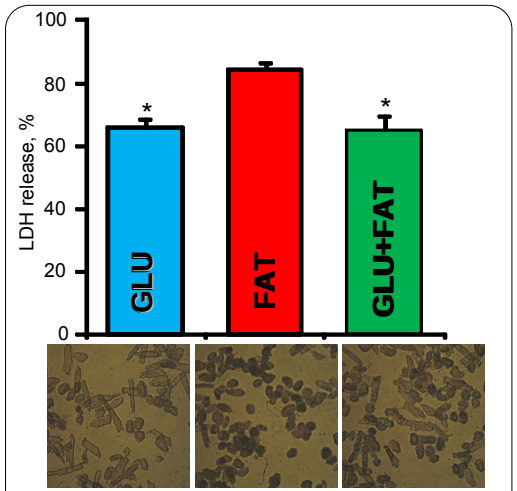


Figure 5. LDH release upon simulated IR injury. ACM were subjected to simulated IR. LDH release (cell death) was expressed as a percentage of total LDH (upon lysis with Triton X-100). Data are the means ± S.E., n = 5 independent plates *, p < 0.05 versus FAT, ANOVA.
Below the graph are representative photomicrographs which show typical morphological changes in cardiomyocytes supplied with either GLU, FAT or GLU+FAT and subjected to simulated IR.

Discussion and Conclusions

- AMC exhibited higher basal oxygen consumption rate but lower uncoupled rate with FAT as a fuel (vs. GLU) (Figure 2). Thus, FAT=smaller metabolic reserve capacity.
- IR shifted cell metabolism toward glycolysis (elevated ECAR) and away from OxPhos (Figure 4). This trend was greater in cells burning FAT.
- AMC had better recovery from IR injury using GLU as fuel (Figure 5).
- Lower cell viability in FAT (vs. GLU) (Figure 5) was correlated with smaller metabolic reserve capacity (Figure 2), smaller ECAR at the pre-ischemic level (Figure 4B) and with a smaller pH drop during ischemia (Figure 3B). This is consistent with a known protective role for acidification during IR injury [3].
- Mixed substrates (GLU+FAT) gave a similar response to glucose alone (Figure 5), suggesting that fat may not be toxic, rather glucose is protective, in IR injury

References: [1] Wojtovich A. et.al. (2013) A non-cardiomyocyte autonomous mechanism of cardioprotection involving the SLO1 BK channel. PeerJ. 1, e48. doi: 10.7717/peerj.48. [2] Guo S et al. (2012) A cell-based phenotypic assay to identify cardioprotective agents. Circ Res. 110, 948-57. [3] Cohen M. et.al. (2007) The pH Hypothesis of Postconditioning. Staccato Reperfusion Reintroduces Oxygen and Perpetuates Myocardial Acidosis. Circulation . 115, 1895-1903 .

Acknowledgements: This work was funded by NIH RO1 HL-071158.